ASPECTS OF DEVELOPMENTAL PATHOLOGY

R.N. LAURINI

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STELLINGEN

I

Bij elk universitair medisch centrum dient een patholoog te zijn aangesteld die deskundig is op het terrein van de ontwikkelingspathologie.

II

Het is van het grootste belang dat post-mortaal onderzoek wordt verricht in elk geval van foetaal, neonataal of post-neonataal overlijden.

III

Voor een kritische beoordeling van de perinatale sterfte is het instituut van de "Perinatale Audit" onmisbaar.

IV

Voor een optimale antenatale diagnostiek en begeleiding is de bijdrage van een ter zake deskundig patholoog onmisbaar.

V

De ontwikkeling van de placenta speelt een belangrijke rol bij het verloop van de zwangerschap bij Diabetes Mellitus.

VI

Zwangerschap bij de rat is een bruikbaar experimenteel model voor het bestuderen van de utero-placentaire circulatie en foetale groeivertraging.

VII

Er dienen bij mamma-carcinoom drie typen van elastose te worden onderscheiden: periductaal, vasculair en interstitieel.

VIII

Het morfologische fenomeen van elastose bij mammacarcinoom heeft geen prognostische betekenis.

IX

Er zijn geen histologische parameters die het toelaten een uitspraak te doen over de prognose van Mola Hydatidosa.

X

Maligne tumoren van het ovarium kunnen PAS-positieve hyaliene insluitsels bevatten die negatief zijn voor alpha-fetoproteine.

XI

Het zogenaamde Multifocale Extra-ovariële Papillaire Oppervlakte Carcinoom ontstaat uit oppervlakte-epitheel met Müllerse differentiatie.

XII

Bij de huidige gemiddelde levensduur van de westerse mens zijn ziektecijfers een betere indicatie voor de kwaliteit van de gezondheidszorg dan sterftecijfers.

Stellingen behorende bij het proefschrift van R.N. Laurini, Groningen 17 december 1986.

RIJKSUNIVERSITEIT TE GRONINGEN

ASPECTS OF DEVELOPMENTAL PATHOLOGY

PROEFSCHRIFT

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. E. Bleumink
in het openbaar te verdedigen op woensdag 17 december 1986
des namiddags te 2.45 uur precies
door

RICARDO NORBERTO LAURINI

geboren te Apóstoles

1986
DRUKKERIJ VAN DENDEREN B.V.
GRONINGEN

Promotor : Prof. Dr. J.D. Elema
Referent : Dr. G.H.A. Visser

To Sidsel
For Nikolas

A falling birth-rate and an increasing interest in antenatal pathology are matters which have come together, not quite fortuitously.

In the second place, the increasing burden, financial and otherwise, upon the state due to the presence in the community of the "unfit" has done something to direct attention more particularly to antenatal pathology.

In the third place, advances in other, but cognate branches of medical and biological science have directed attention to antenatal pathology.

In the fourth place, and finally, it is to be hoped the human desire to carry to the infant yet unborn some of the benefits of modern medicine and hygiene has been and is instrumental in attracting many members of the medical profession to the study of antenatal affairs.

Ballentyne in 1902

Pathologists as a group have been slow to respond with the result that there are few pathologists with strong interests in perinatology. A major purpose of this book is to generate new interest in perinatology among pathologists. The field probably has more unexplored and unsolved problems of clinical significance than any other area of human medicine. A greater participation by pathologists will both accelerate the solving of these problems and provide professional satisfactions to both pathologists and their clinical colleagues.

Richard L. Naeye
John M. Kissane, 1981

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CONTENTS

CHAPTER 1: INTRODUCTION

THE DEVELOPMENT OF DEVELOPMENTAL PATHOLOGY

The last 25 years antenatal diagnosis, high risk obstetrics, neonatal intensive care and genetic counselling have developed as result of the introduction of new techniques such as ultrasound, amniocentesis, respirator treatment etc. This brought a new dimension into both the field of clinical medicine and pathology. The clinical specialities followed this development by creating subspecialities, e.g. neonatology, while in a number of centers perinatal pathology is becoming established as a subdivision of the more traditional field of pathology. Perinatal medicine focuses on the importance of perinatal disease in the context of neonatal survival with initial emphasis on the possible influence of events immediately prior to delivery or during birth. More recent developments in antenetal diagnosis and surveillance highlighten the role played by events during pregnancy and help establish fetal medicine with the inclusion of the earlier stadia of intrauterine life. Moreover the capacity to assess fetal condition and identify fetuses at risk of death or damage in utero allows for a more optimal planning of perinatal care (MANNING 1985).

Morphological examination of spontaneous or elective fetal deaths indicate that, in many cases the damage already occurred during the embryonic and fetal period. Indeed it is most important to bear in mind that today's morphological examination at postmortem is no longer related to an end-stage situation i.e. stillbirth, with a somewhat limited knowledge over the preceding gestational period. Modern prenatal diagnosis allows for the study of fetuses at different stages of gestation accompanied by what can be considered a very detailed "intrauterine clinical history". Furthermore pathologists are not only required to examine fetal deaths but can also be provided with fetal tissue samples following fetoscopy (NICOLAIDES and RODECK 1985).

These developments in the field of fetal medicine have not only evolved from the experimental to the clinical stage but will, no doubt, undergo a more widespread use in the very near future.

The period between conception and 1 year of postnatal life represents a biological continuum where the developmental stage itself is very

much part of the overall picture as are the different forms of fetal and neonatal disease. Furthermore it allows for the study of the sequelae of perinatal disease and intensive care in the socalled "postponed neonatal deaths" (HACK et al. 1980). The preterm baby born at 27 weeks gestation that dies after 5 weeks will be registered as a postneonatal death but the development and associated pathology will still partly be that of 32 weeks gestation. Therefore perinatal pathology has expanded in two directions to cover both embryonic and fetal pathology as well as postneonatal pathology thus evolving into developmental pathology (LARSSEN and LAURINI 1984, LAURINI 1986). From a developmental point of view the changes that occur in the fetal period (8-24 weeks gestation) do so in a stage of tissue and organ differentiation as opposed to the process of organogenesis that dominates the first eight weeks of gestation or to those of intrauterine growth and adaptation to extrauterine conditions that so influence the perinatal period (> 24 weeks gestation) and after. Therefore this author suggests that developmental pathology is divided into embryonic pathology (up to 8 weeks gestation), fetal pathology (8 to 24 weeks gestation), perinatal pathology (25 weeks gestation to 1 week postnatal), neonatal pathology (1 to 4 weeks) and postneonatal pathology over 4 weeks (Fig. 1).

Mainly as a result of the developments around antenatal care it is increasingly recognized that registering and examing products of conception from early pregnancies is of medical and epidemiological importance. These specimens have been traditionally labelled as abortion material and include both first (early abortions) and second trimester (late abortions) pathology. Unfortunately the use of terms like early and late abortions has a very detrimental effect upon the establishment of any form of diagnostic service or registration regarding this material. Therefore one should avoid the use of the term abortion and refer to embryonic pathology (embryo + gestational sac) and fetal pathology (fetus + placenta) since what we are really dealing with are embryonic and fetal deaths which should be considered in a similar way as the neonate-placenta-mother unit of the traditional perinatal period.

In the past the study of human intrauterine development and its deviations was based mainly on the examination of fetal deaths, with limited knowledge on preceding prenatal events. Therefore the interpretation of such postmortem morphology was hampered because these pure

anatomical findings lacked the necessary correlation with in vivo observations to substantiate their real importance.

In summary the extrapolation from necropsy data to conditions during life was not accepted and many a structural change was considered to be the result of postmortem artefact. The introduction of modern ultrasound allows not only for the assessment of fetal anatomy but also of fetal function including fetal behavioural states (TUCK 1986). This has represented a dramatic turn in the approach to the pathology of early pregnancy since now the anatomical findings at postmortem can be correlated to both the intrauterine structural as well as functional status. This dynamic rendering of the study of form is what can be defined as functional morphology.

In conclusion the value of the morphological assessment of embryonic and fetal deaths can be summarized as follows.

1) patient reassurance in cases without significant pathology
2) can demonstrate significant unsuspected abnormalities before the patient undergoes a prolonged reproductive loss. This represents a cost-effective approach that allows for an earlier start of a more comprehensive antenatal care of future pregnancies.
3) it is a valuable diagnostic tool when antenatal diagnosis possibilities are limited and helpful when such studies are unsuccessful.
4) it represents a necessary confirmation of the findings with antenatal diagnosis, including the demonstration of additional findings that can help future antenatal diagnosis and genetic counselling.
5) can help to develop new forms of antenatal diagnosis (i.e. fetal behavioural patterns).
6) can help to develop a better understanding of early human development and its pathology.
7) morphological studies are part of the clinicopathological correlation that will allow for a better understanding of both the etiology and pathogenesis of early human reproductive loss. This is an important step in the establishment of possible therapy or prevention.
8) can help with the epidemiological surveillance of anomalies.

Neonatal pathology and infant pathology are of great importance in relation to the implications of intensive care (mainly in neonatology) and corrective surgery in neonates and infants. This is emphasized by the close collaboration needed between the clinicians performing

echography of central nervous system and surgeons performing surgery on congenital heart disease with the developmental pathologist responsible for developmental neuropathology and morphological studies of congenital heart diseases. Moreover babies, admitted to perinatal intensive units, highlight the importance of iatrogenic pathology as an integral part of the surveillance function of developmental pathology on further development of such diagnostic and therapeutical advances as we experience today in the field of perinatal care.
Furthermore in the context of active obstetrical care, prematurity, intrauterine growth retardation and of intensive neonatal care, the pathology of development must expand to also cover postneonatal pathology in order to ascertain the early sequelae to such an active approach.

Today it is important to include the epidemiological factor into the equation of developmental pathology. Perinatal epidemiology is an established part of the services in perinatal care in advanced international centers mainly through monthly meetings and annual reports on perinatal morbidity and mortality.
This is achieved by establishing a monthly perinatal audit where all the involved disciplines are represented and where developmental pathology plays an important role.
Perinatal epidemiology has recently become a field of interest, not only because of its scientific implications, but also because of its importance in the planning and surveillance of perinatal care.

A SERVICE IN DEVELOPMENTAL PATHOLOGY

All the above mentioned remarks taken into consideration, a service in developmental pathology on a regional basis, attached to the University Hospital, is a must. Such a service must be established in close collaboration with centers for amniocentesis, high risk pregnancy, neonatal intensive care, medical genetics, group for cardiology (cardiosurgery and radiology) and epidemiology.
The necessary workload to make it rational to establish such a service in developmental pathology has, mainly, to be based on the number of postmortems per year since they represent the main part of the workload with the exception of placentas from live-borns and gestational sacs without embryos. An example of such a workload can be better demonstra-

ted on the basis of a population of 500.000 inhabitants which will provide not only sufficient material but also sufficient variation in the material to make such an excercise rational.
Table 1 illustrates the expected workload based on a Dutch population of 500.000 inhabitants. All statistical data correspond to 1984 and was supplied by the Central Bureau of Statistics (The Netherlands) except for the rate of partus immaturus (PUYENBROEK 1979).

TABLE 1

		ABSOLUTE
Livebirths per 1000 inhabitants	12.1	6050
Partus immaturus (17-28 weeks) per 1000 births	7.5	45
Late fetal deaths (≥ 28 weeks) per 1000 births	5.9	36
Perinatal deaths (≥ 28 weeks to 1 week postnatal) per 1000 births	10	61
Early neonatal deaths (first postnatal week) per 1000 livebirths	4.2	25
Neonatal deaths (up to 4th postnatal week) per 1000 livebirths	5.1	31
Postneonatal deaths (> 4 weeks-1 year) per 1000 livebirths	3.2	19
Infant deaths (< 1 year) per 1000 livebirths	8.3	50

On the basis of these figures we see that the expected workload is approximately 131 developmental postmortems. Our experience (see PERINATAL AUDIT IN NORWAY) as well as that of others (MANISCALCO et al. 1982) shows that the perinatal postmortem rate lies around 75% of perinatal deaths. Therefore it is safe to expect around 100 developmental postmortems in the above mentioned population. The workload on placentas can be calculated as that which results from examining all placentas from high risk pregnancies (between 15 and 20% of livebirths) which in our model population could represent about 1000 placentas a year.

Embryonic and fetal deaths are the result from both spontaneous and elective termination of pregnancy. If one defines abortion as a pregnancy loss < 17 weeks gestation, the workload in terms of embryonic and fetal specimens represented approximately 15% in relation to the number of livebirths for the University Hospital in Groningen. Furthermore in 7% of these cases there was a complete embryo/fetus available for postmortem examination. This incidence of abortions is in agreement with HUISJES'S (1984) recent epidemiological review of the subject.
The material consists of complete embryos/fetuses and gestational sacs/placentas or fragmented aborted specimens mainly after vacuum extraction (i.e. following antenatal diagnosis by chorion villous biopsy (CVB).
All specimens are to be send for morphological examination regardless whether they are the result of a spontaneous or elective termination of pregnancy. Established guidelines are to be used for the proper collection of material in order to allow for the use of different methods (i.e. chromosomal analysis, morphology etc.).
Since the majority of cases in the above mentioned age group will be represented by embryonic deaths, the expected yield is approximately a) 80% growth disorganized embryos, b.) 5% of embryos with developmental defects and c.) 15% of normal embryos (KALOUSEK and POLAND 1984). Analysis of this material is essential for proper obstetric and genetic counselling: a) Growth disorganization is usually related to "de novo" severe chromosomal abnormalities without consequences for future pregnancies, unless it happens as a manifestation of recurrent or habitual abortion. Specific developmental defects are an important indication for genetic counselling and close follow-up of a future pregnancy. Normal embryos exclude a number of possible factors and point to a possible hormonal imbalance or infection (KALOUSEK and POLAND 1984). It is also of paramount importance in relation to gestational trophoblastic disease with special emphasis on the relationship between chromosomal abnormalities and the partial mole as well as the complete mole and its relation to invasive mole and choriocarcinoma. The material from both embryonic and perinatal postmortems represent the only opportunity for the study of human development which has become of paramount clinical importance because of the development of intrauterine techniques for obstetrical control and antenatal diagnosis (ultrasound, real time scan, foetoscopy, amniocentesis, placental biopsy etc.) as well as early intervention in

pregnancy including the possibility of intrauterine surgery. Moreover the final expression of fetal disease is significantly dependent on the developmental stage at which the noxious stimulus takes place or of the developmental deragement(s) that follow genetically induced fetal changes.

It is very important that the findings in developmental pathology are regularly discussed at a series of meetings where all the disciplines involved are represented. This entails the establisment of a monthly perinatal audit, regular meetings which the department of medical genetics, the cardiology group for discussion of all postmortems from congenital heart disease, the department of obstetrics and, separately, with the department of paediatrics in order to deal with more specific subjects related to these two specialities that can not be dealt with in the more general context of a perinatal audit.

Other functions to be covered by the developmental pathologist are the establisment of a regular service at peripheral hospitals in the region (taken into consideration the difficulties in centralization of all the material), undergraduate and post-graduate training in paediatric pathology as well as consulting functions from other pathologists in the region.

Such a workload as mentioned above must be covered by one pathologist, two technicians and one secretary. This recommendation is based on international figures, including those from the "EUROCAT-guide to a service in fetal and perinatal pathology" (1981). This author was engaged as one of the consultants responsible for establishing these guidelines. The establisment of such a service in developmental pathology will not only enhance the patient care and research aspects of fetal and perinatal care services but also allow for the training of residents and postgraduate training of pathologists in this field. This is a necessary development in as much as developmental pathology has become part of the daily patient care routine at both university and non-university hospital. The developments in the field of obstetrics, neonatology (probably better defined as developmental medicine), and genetics together with social changes put an increasing pressure on the pathologist establishing the need for expertise in diagnostic developmental pathology as required for other more traditional disciplines i.e. cancer diagnosis.

As a matter of fact there is already indication that careless handling

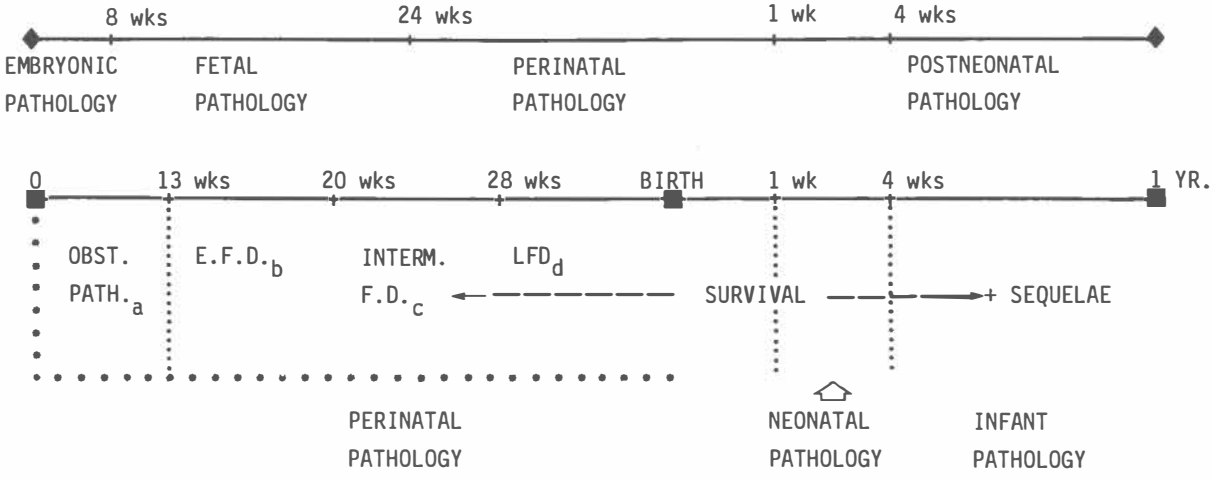
of such material without the routine rigour of surgical pathology can be considered negligent and become liable to prosecution (MILUNSKY 1983).

OUTLINE

It is the aim of this thesis to establish guidelines for a service in developmental pathology that will further enhance the different aspects of perinatal medicine.
The long-time experience with some special techniques of postmortem examination of embryos, fetuses and infants is reported (chapter 2) as well as the results of a number of studies in different areas of developmental pathology (chapter 3, 4, 5 and 6), some of which have already been published. In chapter 7 the results of an experimental study on the effect of diabetes on fetus and placenta are reported.
This author was responsible for the pathological expertise in the panel that carried out the Scandinavian studies on perinatal epidemiology (chapter 3).

FIGURE 1 *

DEVELOPMENTAL PATHOLOGY



a OBSTETRICAL PATHOLOGY •

b EARLY FETAL DEATHS

c INTERMEDIATE FETAL DEATHS

d LATE FETAL DEATHS

* Modified from Laurini (1986)

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CHAPTER 2: METHODS IN DEVELOPMENTAL PATHOLOGY

2.1. DEVELOPMENTAL POSTMORTEM

Experience from different countries shows that many perinatal postmortems are too incomplete to provide data necessary for eventual obstetric and genetic counselling, guidance of antenatal care in future pregnancies or use in epidemiological studies (BARSON 1981). A perinatal postmortem protocol must aim not only at the study of eventual pathology, but also at the assessment of development, intensive care associated changes, risk factors for future pregnancies, fetal morphology in relation to intrauterine intervention and epidemiological aspects.
In addition it must also provide with documentation for clinicopathological conferences and for any eventual future review as, for example, for genetic counselling.
In the Introduction (Chapter 1) developmental pathology was divided into different periods (Fig. 1). Except for embryonic deaths all other specimens are dealt with following a developmental postmortem protocol.

As a comprehensive information on methods in developmental pathology is scarse, the subject is dealt with in some detail in this chapter.

EMBRYONIC DEATHS

The apparent limited diagnostic value of first trimester specimens is the result of an insufficient examination that usually only provides the clinician with a further confirmation of pregnancy.
In order to correct this deficiency a first step is to request to the clinicians that the whole specimen is sent instead of a selection of the products of conception. Furthermore these specimens must undergo a careful gross examination of the whole specimen in a petri-glass after proper rinsing in water. This procedure will allow for the diagnosis of developmental stage, presence of congenital abnormalities and identification of the different structures that must be routinely sampled for histological examination (embryo, umbilical cord, membranes, yolk sac, placenta (including vesicles) and decidua).

The types of congenital anomalies are similar to those seen at

later stages (POLAND 1968) but one must pay special attention to development inconsistencies that serve as indicators for genetic counselling (KALOUSEK and POLAND 1984).

Specimens with a crown-rump length of up to 3 to 4 cm are routinely examined by means of a set of standard planes of transversal sections (Appendix 1). For the slicing of these specimens a razor blade is used and, when macerated, the routine hematoxylin-eosin staining is replaced by a trichrome stain (i.e. Masson). Especially with the advent of the chorion villous biopsy and consequent early intervention the specimens consist of fragments formed by fetal parts together with placenta and decidua. A complete postmortem examination is to be carried out regardless whether the specimens are complete or fragmented. The appendix provides with a form (Appendix 2) used for the examination of first trimester specimens and includes a suggested working classification based on six groups (Appendix 3). Furthermore there is also a morphological classification of gestational trophoblastic disease (Appendix 4) with comments on the parameters to assess (ELSTON 1982, personal communication).

DEVELOPMENTAL POSTMORTEM PROTOCOL

For the purposes of this chapter all autopsies on cases from 12 weeks gestation onwards are treated as developmental postmortems. In case of intrauterine death or elective termination of pregnancy both the fetus and the placenta must be sent unfixed to the pathology department. Neonatal deaths must be sent with all catheters, drainages etc. in situ. These must be examined in situ by the pathologist after the postmortem X-ray examination has been carried out. In case an intrauterine or neonatal death is to be sent from outside the hospital, it is recommended that the physician in charge of the case contacts the pathology department on before hand in order to make the necessary arrangements with regard to eventual supplementary studies that need to be done immediately and, further, to agree on how to send the material.

CLINICAL INFORMATION

As for any type of postmortem examination, it is important to first

go through the clinical notes. A comprehensive family history, together with information on pregnancy, delivery and neonatal events is necessary to plan the postmortem and assess the findings. It is not uncommon that clinical notes are not available or lack notes on terminal events. The physicians in charge of the case are not always able to attend the postmortem. It is therefore recommended that a request form is used that will provide the pathologist with the necessary clinical data (Appendix 5). At this stage the pathologist must plan the possible supplementary investigations needed for the individual case.

EXTERNAL EXAMINATION

X-ray examination is today mandatory and will be dealt with separately.

Photographic records are necessary to accurately document congenital malformations and abnormal phenotypes. Whole-body pictures (anterior-side-posterior) as well as close-up of selected features are recommended. A Polaroid-camera can be used when a photographer is not available. This objective documentation proves very helpful for any later review of the findings, for instance in case of genetic counselling.

Supplementary investigations are used as in clinical work. These ancillary tests must be selected in close collaboration with the clinicians to ensure that the necessary tests are performed as well as to avoid requesting tests twice that represents an undesirable extra workload for other laboratories.

Hydrops foetalis is a good example of the importance of planning before proceeding with the postmortem (MACHIN 1981).

Cytogenetic investigations are today considered an important part of the perinatal postmortem. Still there is a need to be selective. This method is most useful for perinatal deaths under 28 weeks gestation, in the presence of multiple malformations and for macerated fetuses (ALBERMAN and CREASY 1977). For chromosomal analysis in cases of macerated fetuses, it is recommended to take amnion for amniotic cells survive several days after fetal death (EUROCAT GUIDE 1981). The perinatal postmortem also represents an important tool in the diagnosis of metabolic disorders. Biochemical investigations can be necessary to either establish a diagnosis or to confirm a prenatal diagnosis

of a metabolic defect (ANONYMOUS EDITORIAL, LANCET 1984).

The anthropometric assessment is an important part of the perinatal postmortem examination (see 2.4). The necessary data is listed in Appendix 6. Chest circumference is measured at the nipples and abdominal circumference at the umbilicus.

INTERNAL EXAMINATION

It is of practical consequence to bear in mind that any postmortem protocol should be rigidly followed, but must allow for necessary flexibility according to the need of the individual cases.

One starts the postmortem with a thorough study of the organs in situ. This allows for assessment of organs' position, relations, connections and development. The following step is to take out the organs in the form of neck/ thorax block, abdominal block and retroperitoneal/ pelvis block. These blocks are fixed for one to two days before further dissection. The weights of the organs are taken after dissection. The pathologist should personally remove the central nervous system (CNS) following routine methods that will be described separately.

The heart-lung block requires special attention to deal with the possible presence of cardiovascular anomalies. The heart-lung preparation must not be separated from the rest until all vascular connections have been examined. Thus infradiaphragmatic pulmonary drainage in cases of total anomalous venous return is not missed or damaged in a way that makes reconstruction of the situation impossible. Furthermore, the heart-lung block must be preserved intact until complete dissection is performed for the study of eventual congenital heart malformations as well as to assess any surgical correction (see Appendix 7).

It is generally believed that postmortem examination of macerated cases gives very little information. Still, experience with this type of material demonstrates that valuable information can be obtained through unexpected and significant findings among these postmortems (BECKER 1976, EUROCAT GUIDE 1981). Macerated intrauterine deaths are examined following the same protocol as for any other perinatal postmortem. In cases with severe maceration, it is recommended to inspect the CNS in situ because of the difficulty in handling. In macerated smaller fetuses, the CNS can be fixed in situ before attempting removal.

HISTOLOGIC EXAMINATION

A complete histologic examination is carried out for all cases with a conceptional age up to 42 weeks regardless of the macroscopic findings. In the perinatal period, anatomical (pathological) findings are dependent on a standardized histologic examination since gross examination can give a false impression of normality. Such detailed histology is needed both for the diagnosis of pathology and the assessment of maturity.

Adaptation to extrauterine life, disease patterns and intensive care associated changes are closely related to the maturity of the individual cases. Estimation of maturity is also useful and necessary in assessing macerated fetal deaths. In these cases better results are obtained by replacing haematoxylin-eosin with a trichrome stain.

When there are no changes on gross examination routine blocks are taken from each organ (Appendix 8). It is best to avoid the use of sonde or clipping of structures before blocking them, i.e. block from trachea-oesophagus-thyroid or ductus arteriosus. In the presence of pathology samples are taken both from normal and abnormal areas.

At least one block is taken from each lung lobe as well as from each intestinal segment including the appendix. Routine heart histology consists of representative tissue samples from right, left ventricle and septum. The ureter-aorta-vena cava block is taken at the level of the bifurcation of the aorta. The "cloaca" block represents a saggital cut through the urethra to display urogenital structures and rectum. Diagnosis of sexual phenotype includes samples from all internal genital organs. The assessment of the lymphoid tissue is carried out on tissue samples from thymus, spleen, intestine and lymph nodes present in mesenterium and axillary fat. Routine bone marrow specimen is taken from femur. To avoid trauma, it is best to take the hypophysis surrounded by the cartilagenous part of the sella turcica. In smaller fetuses blocks from the spinal cord are taken together with surrounding vertebral structures. The 5th costochondral junction is routinely blocked for assessment of intrauterine growth (EMERY and KALPAKTSOGLOU 1967).

Gross examination in cases above 42 weeks conceptional age yield a more complete picture. Therefore an experienced pathologist can be more selective with regard to histology and X-ray examination.

PLACENTA

A complete perinatal postmortem examination must include the placenta. Pathologists should make the necessary arrangements with the local maternity centers to ensure that all intrauterine deaths are sent with the placenta. When available, the placenta should also follow cases of transport to a neonatal unit. The placentas should be sent FRESH to the local department of pathology. Placentas from night and week-end deliveries are to be kept UNFIXED in a plastic bag (properly identified) at $4^{o}C$ (refrigerator) and delivered to the pathology department in the morning of the first working day. If necessary placentas can be kept this way for up to 3 days i.e. a placenta delivered Friday night can be so kept until it is sent to the pathology department on Monday morning.

The placenta is to be examined not earlier than 4 to 6 hours after delivery to allow for blood drainage. The placenta is to be weighed after trimming the umbilical cord and membranes as well as removing all clotted blood from the maternal surface. The placenta can then be fixed in formalin and further examined by coronal sections (1 cm thick) after 72 hours fixation. Any abnormalities seen on gross examination are to be expressed in estimated percentages of the total placental volume.

The following standard samples are to be taken from all placentas: A) umbilical cord, B) a "roll" of the membranes, C) site of umbilical insertion (u.c.), D) 2 central samples through the cotyledon center (see entrance spiral artery into cotyledon on the maternal surface) from a macroscopically normal placenta. If the placenta is more than 2 cm thick, divide the block into 2 halves, E) one selected sample from predominant pathology or unusual features (Appendix 8).

CONCLUSION

When proceeding to summarize a case, one must analyse the findings to establish (a) developmental stage, (b) eventual pathology, (c) possible relation between developmental stage (grade) and pathology, (d) eventual iatrogenic disease, and (e) risk factors (genetic/non-genetic) for future pregnancies and/or relative (Appendix 9). The

assessment of pathology has much in common with general pathology. Still a very important difference is that eventual pathology must always be worded in the context of the maturity (developmental stage) of the individual case.
This also applies for the assessment of changes as result of neonatal intensive care or iatrogenic disease (KEELING 1981). Causes of perinatal mortality can be difficult to establish and many intrauterine deaths go unaccounted for. A pathologist with an interest form as well as experience with perinatal pathology will be able to elucidate a majority of cases. Still perinatal mortality is often multifactorial in origin. Only a close collaboration between obstetricians, neonatologists, genetecists and pathologists will allow for any substantial progress in the field of perinatal care.

2.2. ROUTINE POSTMORTEM RADIOGRAPHY

Radiography, as part of a perinatal postmortem protocol, is a simple and informative method that provides with information for all the above mentioned aims. Many pathologic conditions can be detected, and the postmortem planned accordingly (LAURINI and GROOTE 1984). Moreover radiography is important for the assessment of growth and maturation. Therefore X-ray examination has been included in the perinatal postmortem protocol of a number of centers including ours. We photograph all cases between 12 weeks gestation and 42 weeks postconceptional age (gestational age + postnatal age). Routine radiography consists of an anteroposterior and lateral view of the entire body. Additional exposures of individual parts, films of dissected structures or contrast studies are performed only when indicated. A Faxitron X-ray machine (Faxitron Series - Hewlett Packard) is used for cases with a crown-heel length of up to 38 cm. For babies with a crown-heel length above 38 cm one can establish a collaboration with the X-ray department.
For the anteroposterior view a roll of low density material (i.e. paper or cottonwool) must be placed under the neck to slightly extend as well as stabilize the head in a centered position. The upper extremities should be at the sides, flexed at the elbows and with extended

fingers. The lower extremities are positioned in abduction with knees flat on the film. To fit the larger babies onto the mammography film (24 x 30 cm) we flex the lower extremities. For the lateral film it is important to extend the arms in front of the chest together with legs flexed in different angles at the hip in order to avoid superposition of skeletal structures. Tapes can be used to maintain the desired positions. X-ray examinations are to be performed with all catheters etc. in situ. The film is to be covered with a plastic sheet to avoid the wet surface of the fetus coming in contact with the film thereby producing artefacts.
For the Faxitron we have established a standard exposure protocol (Table 1) based on the use of a mammography film (mammary RP3).

TABLE 1. Exposure protocol.

Crown-heel (cm)	Gestational age (weeks)	Kilovoltage	Time (min)
5	(11)	20	3
6/9	(11.5-12)	22.5	3
10/13	(12.5-15)	25	3
14/20	(15-18.5)	30	3
21/27	(19-22.5)	35	3
28/33	(23-24)	40	3
34/45	(25-34)	45	3

We have also used screen films and, in our experience, there is a distinct advantage in the use of mammography films. This expressly applies to the cases examined with the Faxitron.
The routine films are examined before proceeding with the autopsy so that the postmortem examination can be planned accordingly. X-rays of the skeletal system can, if necessary, be obtained after thoracoabdominal eviceration but such an approach has obvious limitations. Additional exposures of individual structures are especially useful for head (i.e. holoprosencephaly) and extremities (i.e. chondrodystrophia punctata). Otherwise, mainly in the context of skeletal dysplasias, it is recommended to obtain further views of separate bones (e.g. femur) after dissection. Contrast studies can be performed in situ

(e.g. urinary systems in prune belly syndrome etc.) or after dissection (e.g. postmortem angiocardiography in congenital heart diseases). Water-soluble agents are quite suitable for these examinations. We have recently carried out a review of 212 consecutive cases from our files and the most important findings are summarized in Table 2.

TABLE 2. Radiographic findings in 212 cases*.

	N	%
Cranio-spinal anomalies	12	7.3
Skeletal dysplasias and other skeletal anomalies	18	10.9
Pathological calcifications	4	2.4
Growth deviations	30	18.2
Gas in abnormal sites	11	6.7
Hydrops foetalis	5	3
Lung hypoplasia	21	12.7
Other soft tissue abnormalities	43	26.1
Hydrocephaly	4	2.4
Microcephaly	4	2.4
Iatrogenic abnormalities	5	3
Miscellaneous	8	4.9
No abnormality	94	44

* A case might show more than one abnormality

In this material 118 cases (56%) depicted diagnostically useful abnormalities including 31 instances (15%) which represented conditions that might otherwise have been undetected at postmortem (Table 3). The most common findings that might have escaped detection at autopsy were mainly those of skeletal abnormalities associated to neural tube defects (i.e. malformed ribs, hemivertebras) or isolated findings as variation in the number of ossified ribs.
Gas in abnormal sites included pneumothorax (n=3), pneumoperitoneum (n=1) and intravascular gas in stillbirth (n=1). Radiography provides the only reliable method for detection of air collections at autopsy.

TABLE 3. Conditions otherwise easily missed at postmortem.

Craniospinal anomalies	1
Other skeletal anomalies	16
Skeletal dysplasia	2
Pathological calcifications	2
Gas in abnormal sites	5
Iatrogenic abnormalities	3
Miscellaneous	2

Moreover a pneumothorax may be a major contributing factor in the cause of death of neonates being managed with assisted ventilation. Therefore even if a chest X-ray is available from soon before death, it is recommended that the postmortem X-ray examination is carried out because the development of a significant pneumothorax might be an acute terminal event that precipitates the death of the patient. Iatrogenic abnormalities were represented by lesions following catheterization. An example of this was traumatic non-bacterial thrombotic endocarditis around the fossa ovalis or septum resulting from a misplaced umbilical vein catheter well into the right atrium, as seen in two cases (SYMCHYCH et al. 1977). The position of the tip of the catheter on the radiograph alerts the pathologist who proceeds to open the right atrium with the catheter in situ. This procedure allows for the recognition of small and friable thrombi on the catheter and, usually, around the fossa ovalis or septum.

There were two cases of skeletal dysplasia: dyssegmental dwarfism (see Chapter 4) and thanatophoric dysplasia. The latter also represented an example of the use of postmortem radiography in cases where permission for postmortem is refused. In this case although a postmortem was not done because of religious reasons, the radiological examination allowed for the diagnosis of thanatophoric dysplasia. This was of help in counselling this couple who expressed the wish to have more children. In a series of 250 radiographs of stillborn and neonatal deaths BARSON et al. (1974) reported that 40% showed significant radiographical pathology. The author did not refer, however, to the assessment of growth and growth deviations. The findings listed in Table 2 are

similar to those reported by FOOTE et al. (1978) in a review of 2500 routine postmortem radiographic examination of perinatal cases. The complex skeletal and soft tissue abnormalities associated with different types of neural tube defect can only be documented by X-ray examinations. As for a number of other malformations, radiography will provide a more comprehensive assessment, vital to further counselling. This is even more so in cases of skeletal dysplasias where X-ray can be the most important diagnostic tool (e.g. osteogenesis imperfecta). Abdominal calcifications can be seen in cases of meconium peritonitis in mucoviscidosis. Still we have also observed similar changes as a result of calcified intraluminal meconium in severely macerated retained fetuses or as a result of dystrophic calcification in areas of tissue necrosis following intrauterine ischaemic changes.

The assessment of soft tissue conditions is as important as that of the skeletal structures. Conditions like hyaline membrane disease and interstitial emphysema can be easily identified. Hernias (e.g. diaphragmatic), tumours (e.g. cervical teratoma), and different forms of abdominal distention (e.g. prune belly) can be depicted and will allow for a better planning of the postmortem procedures.
Hygromas are not only diagnosed but can be further divided into the colli and cervicis type (BERANT et al. 1981). Although a hygroma cervicis in a female fetus strongly points to a Turner syndrome, other anomalies and chromosomal abnormalities must be considered (CHERVENAK et al. 1983).

Both for skeletal and soft tissue conditions, useful comparison can be carried out with available ultrasound findings. X-ray examination is also useful in the assessment of intensive care associated changes. Among others it can demonstrate misplacement of catheters (e.g. kebab lung) or draw attention to possible lesions as result of trauma by the catheters.
Assessment of mega- and microcephaly will also benefit from the use of postmortem radiography, since standard curves are available both for biparietal and occipitofrontal X-ray diametres (HODGES 1937).

The estimation of maturation is an integral part of the developmental postmortem and radiography is an important tool. This is carried out by the use of a standard curve for the length of the calcified portion of the femur (Appendix 13) as well as with the established patterns

of ossification for knee and heel centers (RUSSELL 1981). More detailed information on estimation of maturation is given in 2.4.

In conclusion we recommend that X-ray examination should be part of a perinatal postmortem protocol for it provides with valuable information on different aspects of the developmental postmortem (LAURINI and GROOTE 1985). It assists mainly in the evaluation of pathology as well as in the assessment of growth and maturation.

2.3. NEUROPATHOLOGICAL EXAMINATION

The final aim of a fetal and neonatal neuropathological examination is to assess the findings in the context of the degree of maturation of the individual case and to attempt to differentiate between a primary maldevelopment from a secondary destructive lesion. This can be carried out only if one follows a comprehensive standard protocol consisting of a detailed gross examination combined with the absolutely necessary histological evaluation. WIGGLESWORTH (1984) has summarized the main guidelines for the postmortem examination of the brain and spinal cord, including the removal of the brain in macerated stillbirths and in cases of hydrocephaly or large brain cysts. Still it is worthwhile to remember that there are several techniques available for the removal of the perinatal CNS (TOWBIN 1970) including the fetal brain (RUDELLI et al. 1983).

The selection of a technique and any eventual modification depends on the clinical data and postmortem X-ray findings for the individual case.

For example when there is doubt about a tentorium tear or any other anatomical abnormality around the confluence of the dural sinuses or in the posterior fossa, it is helpful to remove the brain in three steps. After opening the skull one proceeds to incise the corpus callosum in its total length in order to be able to separate the brain hemisphere. The pedunculi cerebri are then cut at the level of the lamina quadrigemina. It is now possible to remove one brain hemisphere. The same procedure is carried out for the other side. This allows for a detailed examination, in the first instance, of the falx, tentorium and sinuses followed by a closer look into the posterior fossa.

In those cases where there is an indication of increased intracranial pressure, or other causes of coning, possible subdural or subarachnoidal haemorrhage and for collecting sterile cerebro-spinal fluid, the posterior examination of the cisterna magna should be done after the removal of the atlas arch.

It is quite common to be confronted with rather soft brains due to either early gestation or associated pathology. In these cases the actual removal of the brain is carried out in a pre-weighed wide-mouthed water-filled container. The water is later replaced by the fixative.

It is recommended that all perinatal CNS are fixed before they are further examined. The fixative of choice is 10% buffered formalin and optimal fixation can be achieved when the CNS is left in a clean fixative without blood contamination. It is preferable to float the brain in a solution of 10% buffered formalin saturated with NaCl instead of suspending it from the bassilar artery. The brain is weighed after fixation since the increase of negligible and it is preferable to limit, as much as possible, all handling of the unfixed specimen. There is no real advantage in prolonging the fixation time in formalin beyond 3 weeks. Nevertheless further fixation for 1 week in 70% alcohol does significantly improve the handling of the brain, allowing for better slicing and eventual photography (Appendix 10). After fixation the brain hemispheres are removed from the brainstem by cutting the cerebral penducles right under the mamillary bodies. If not already done so the hemispheres are then separated from one another by an incision through the corpus callosum and third ventricle. In most cases each half is divided into two by means of a coronal section through the mamillary bodies. At this stage the 3 routine blocks (motor cortex-white matter, lateral ventricle-subependymal germinal matrix-basal ganglia-thalamus, hippocampus) are taken. Each half of the brain hemispheres is then sectioned following a sagittal plane along the lateral ventricles.

Routine histological examination of the perinatal CNS should be done in all cases. Among others, diffuse anoxic neuronal changes and multiple small areas of periventricular leucomalacia (PVL) are not usually evident on gross examination. Furthermore the microscopic examination is important in the evaluation of the degree of maturation.

It is advisable to carry out such an examination on standard blocks

from selected areas that are both helpful for estimation of development and represents target areas for hypoxic-ischaemic associated changes. Appendix 11 summarizes the routine standard tissue samples necessary for the histological examination of the perinatal CNS. When possible whole brain histological sections from celloidin embedded material are useful (YAKOLEV 1970). Still, for routine diagnostic purposes embedding in a paraffin mixture (80% paraffin, 17.5% stearin, 2.5% bee wax) allows for a less cumbersome processing and staining of whole brain sections. In addition blocks are to be taken from any lesions noted on gross examination. It is important to pay especial attention to the incision of the cerebral peduncles when separating the brainstem and cerebellum from the rest of the brain. The peduncles must be cut right under the mamillary bodies with a small blade scalpel. If we then further cut the brain stem following a horizontal line between the upper border of the pons and the lower end of the colliculus inferior, we obtain the block necessary for the histological examination of the aqueductum, including its narrowest point. This becomes especially useful when assessing a case of hydrocephalus.

EMERY and STASCHAK (1972) have studied the size and form of the cerebral aqueduct in children. Their results indicate that, on fixed material, a child's aqueduct at its narrowest point (first constriction) must be below a diameter of 0.15 mm^2 before it can be considered abnormally narrow.

Processing all these tissue samples requires special handling in order to achieve the necessary quality for the histological evaluation of the subtle changes present in the perinatal CNS. An example of one such method is summarized in Appendix 10.

Examination of the middle and inner ear in the neonate can also provide the pathologist with useful information. The technique for dissection and examination of the middle and inner ear has been recently summarized by KELEHAN (1984).

The ever increasing number of congenital myopathies as well as the need for a differential diagnosis with neurogenic muscle disease has further emphasized the need for morphological and morphometrical assessment of the perinatal muscle. Therefore, at least in cases with a suggestive clinical/genetic history of muscle disease, it is recommended that samples from biceps and quadriceps are frozen in

isopentane and cryostat sections are prepared using the standard staining and enzyme histochemical techniques. SCHLOON et al. (1979) report that the time between death and postmortem did not influence the quality of histochemical reactions in their series. In this author's experience the results with enzyme histochemistry becomes difficult to interprete when more than 12 hours have elapsed between death and postmortem.

Vascular lesions although uncommon, can be seen or suspected in both fetal and neonatal periods. In such cases the cerebral vessels can be examined following the method put forward by NORMAN (1978).

A detailed developmental neuropathology examination as herewith outlined will not only significantly add to the identification and grading of lesions, but also demonstrate that brain pathology associated with acute and/or chronic hypoxic-ischaemic brain damage or infections is usually much more widespread than expected from brain scans and gross examination of the perinatal brain.

2.4. ASSESSMENT OF MATURATION AND GROWTH

The assessment of maturation at postmortem defines the observed degree of development in terms of gestational weeks. The evaluation of growth determines whether growing has been normal or abnormal for the previously established developmental stage (maturation).

Maturation and growth represent critical aspects of human development and developmental pathology. The expression of fetal and neonatal disease is dependent on the stage of maturation and deviations of growth clearly define a high risk population for both hypoxic-ischaemic damage, congenital malformations and chromosomal pathology.

The clinical assessment of duration of pregnancy is based mainly on the first day of the last menstruation (LMP) and/or determination by echography. Quite often however this information is not available, unreliable or subject to influence from maternal/fetal disease. Therefore postmortem parameters must be established for assessment of maturation as well as growth. When possible these must be the equivalent of those used for clinical antenatal assessment.

The aim is to compare the degree of maturation and growth at post-

mortem with that established by the obstetrician and neonatologist in order to interprete the findings in relation to the developmental stage and growth. Such an evaluation is also necessary for the assessment of developmental and growth deviation of separate organs (i.e. lung relative immaturity (maturation) and lung hypoplasia (growth) in oligohydramnios sequence).

MATURATION

We studied the assessment of maturation in 150 cases of fetal and neonatal postmortems in which 6 anthropometric parameters were available and assessed using ossification centres (RUSSELL 1981) and femur length (HODGES 1937) by use of X-ray, foot length (POTTER and CRAIG 1975), development of kidney and brain (DOROVIN-ZIS and DOLMAN 1977) by the use of histology and first day of last menstruation data. A case was included when at least 3 of the 6 parameters agreed on a gestational age (maturation) in weeks that was considered representative for the case. Others have also derived gestational age from the combination of parameters rather than arbitrarily assume that one parameter is a better indicator of gestational age than the rest (JEANTY et al. 1984). The mean $\pm$ 2 SD was calculated for the values in millimeters from the calcified portion of the femur and humerus, the foot length and of the maximum diameter of the thoraxic cage at the level of T5 (vertebra). Smoothed values were obtained using an optimal seven-term formula (POLLARD 1977) (Appendix 13/14/15/16).

Table 4 shows that the gestational ages derived from the measurement of the femur length in postmortem radiograph are in agreement with those derived from ultrasound measurement of the femur length (as discussed by JEANTY et al. 1984, and WARDA et al. 1985). This applies also for the humerus. Furthermore the range of gestational ages derived from the 2 SD of the mean for the femur and humerus (radiographs) corresponds with the estimated 5th and 95th percentile after ultrasound measurements (JEANTY et al. 1984). For the assessment of the T5 only the 78 cases without chest/lung pathology were used.

On the basis of these results guidelines were established for the assessment of maturation from the 12th week of gestation (Appendix 17).

In general it is recommended to use femur length up to 22 weeks, the number of glomerular layers up to 33 weeks, the modifications in the nephrogenic zone for the period between 34 and 36 weeks and the assessment of ossification centres for the time beyond 36 weeks. Nevertheless it is stressed that the assessment of maturation must never be based on only one parameter. From a practical point of view the assessment of developmental stage must comprise at least the femur length (X-ray) (Appendix 13), brain gyral pattern (gross examination) (Appendix 18) and renal development (histology) (DOROVINI-ZIS and DOLMAN 1977). The latter represents the real conceptional age since renal development is not affected by eventual pathology (NAEYE 1975) with the exception of chromosomal abnormalities (see Chapter 4) and renal anomalies (i.e. cystic disease). Both skeletal maturation and brain development can be affected by the presence of pathology. This can be further illustrated by the fact that in intrauterine growth retardation, the assessment of renal development will agree with the conceptional age while the degree of skeletal maturation as

TABLE 4. Gestational ages (weeks) derived from femur length in the present study (postmortem data) and published series (ultrasound).

BONE LENGTH (mm)	PRESENT[1] STUDY	WARDA[2] et al.	HÖHLER[3] and QUETEL	JEANTY[3] et al.	HÖHLER[3] and QUETEL	O'BRIAN[3] et al.	HADLOCK[3] et al.
10	13	13.2	13.3	12.6	12	12	12.8
20	16	16.2	15.3	15.9	15.2	15.2	15.7
30	19	19.7	18.1	19.4	18.6	18.4	18.9
40	24	23.5	21.5	23.1	22.4	21.6	22.5
50	28	27.6	25.8	27.0	26.5	-	26.5
60	32	31.9	30.7	31.1	31.0	-	30.9
70	37	36.4	36.4	35.4	35.7	-	35.7
80	40	40.8	42.8	40.0	40.8	-	40.4

1 derived from Appendix 13 (femur length measured radiographically).

2 WARDA et al. (1985)

3 as discussed by JEANTY et al. (1984)

expressed by the femur length will be equivalent to a shorter gestation. The latter observation is further confirmed by the findings on ultrasound assessment of femur length in intrauterine growth retardation (O'BRIEN and QUEENAN 1982). Hypertensive disorders of pregnancy accelerate fetal cerebral maturation (HADI 1984).

GROWTH

The most common deviation of fetal growth is that of intrauterine growth retardation (I.U.G.R.) (SEEDS 1984). There has been a clear increase in its incidence in the last decade (most probably due to better registration) and today it is associated with approximately 25% of perinatal deaths as shown by several perinatal audits (Chapter 3). I.U.G.R. can be the result of environmental or genetic factors as well as of a combination of these. The environmental factors are represented by maternal conditions (vascular supply-infections-teratogens, etc.) and fetal conditions (congenital anomalies - chromosomal abnormalities, etc.) including the placenta as a fetal organ. In regard to genetic factor there is a need to further understand the growth pattern as result of genotype as opposed to deviations due to genetic disease.

The study of the deviations of fetal growth must not be limited to I.U.G.R. but also include the large for gestational age cases (macrosomia) which also represent a group with increased perinatal risk.

USHER and MCLEAN (1969) have published percentile tables that allow for several anthropometric measurements to be plotted against both gestational age and birth weight. Birth weight, cranium-heel, distance and, head- and chest circumference are compared with standard tables. These tables can be used for fetuses and neonates between 25 and 43 weeks gestation. For cases with a gestational age under 25 weeks, it is recommended to use Potter's (1975) table for body length, body weight and main organ weights. Larroche's (1977) tables are used for assessment of organ weights for cases with a gestational age of 26 weeks or more.
Other useful publications on anthropometric assessment of embryos and early fetuses are: TANIMURA et al. (1971), GOLBUS and

BERRY 81976), MOORE et al. (1981) and HERN (1984).

These anthropometric findings are used to assess fetal growth and classify eventual growth retardation. Values from crown-heel, occipito-frontal circumference (OFC) and head-chest circumference difference are plotted against birthweight (BW) in order to further classify I.U.G.R. into symmetrical (proportionate small for dates baby with BW < 10th percentile for gestational age (GA) and OFC-head/chest difference inside 97th/3rd percentile for BW) and asymmetrical (dysmature baby with BW < 10th percentile for GA and OFC-head/chest difference above 97th percentile for BW) (LAURINI and LARSSEN 1984). Such a classification is also possible during ultrasound examination (KURJAK et al. 1978). The symmetrical type is seen mainly with chromosomal abnormalities, congenital anomalies and infections and can be present before 30/32 weeks gestation. Asymmetrical type is seen with later development of I.U.G.R. (after 30/32 weeks gestation) and is usually associated with extensive placenta infactions and/or maternal disorders as pregnancy induced hypertension.

CONCLUSION

In this chapter guidelines are established for the examination of embryonic and perinatal deaths. The most important aspects as the developmental postmortem, postmortem routine radiography, developmental neuropathology and assessment of maturation and growth are discussed in relation to methodology and results. The advantages of the routine use of such a comprehensive approach will be dealt with in the following chapters.

Nevertheless there is an unfortunate tendency to introduce protocols for the investigation of perinatal deaths that are limited to the recognition of genetic disease (KRONICK et al. 1983, MUELLER et al. 1983). Present changes in obstetrical and neonatal clinical practice highlight the need for a careful appraisal of non-genetic disease. At a time when most pathology departments are somewhat reluctant to adapt to these changes in clinical practice suggestions like that of MUELLER et al. (1984) delay the necessary establishment of a service in developmental pathology that is dependent on the introduction of a methodological approach as discussed in this chapter.

One cannot but agree with NAEYE (1983) that limitations in the routine morphological examination of perinatal deaths can only result in a delay of our understanding of perinatal disease.

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CHAPTER 3: PERINATAL EPIDEMIOLOGY

The aim of those involved in perinatal medicine must be to find ways of reducing perinatal morbidity and mortality by improving the perinatal services. To succeed, one must analyse the causes of perinatal morbidity and mortality in order to influence management. To achieve an improvement in survival one must define those aspects of perinatal mortality that can be influenced by management.
The perinatal audit is an important instrument to study the causes of death as well as identify factors influencing current perinatal mortality.

3.1. PERINATAL AUDIT IN NORWAY

This summarizes the results of a perinatal audit in which the author participated as the responsible pathologist and which was based on all perinatal deaths in five selected Norwegian counties during a 12 month period 1979/1980 (LARSSEN et al. 1982).

METHODS

During this period there were 19,970 births or 38,3 per cent of the annual number of births in Norway. Among these births there were 270 perinatal deaths, including fetal deaths aged 24 weeks or more and neonatal deaths that occurred before the newborn babies were discharged from the maternity institution or neonatal nursery (autopsy rate 75%). Five perinatal deaths which occurred among babies delivered at home or during transportation were not included in the study.
Information on the type of postmortem performed is given in Table 1. In 59 instances the postmortem was done by the author, reports on the remaining ones, performed elsewhere, were reviewed. The panel considered that in 97 cases (48%) the postmortem findings represented a major contribution to the final assessment of these cases. Figure 1 shows that these cases include the most complete postmortems (type 1 and 2) while incomplete ones (type 4 and 5) had little or nothing to add to the evaluation of a case.

TABLE 1. Type of postmortem.

TYPE OF POSTMORTEM	NUMBER	PER CENT
1. Complete perinatal postmortem with X-ray examination	28	13.8
2. Complete gross examination with histology of CNS and some organs	31	15.3
3. Complete gross examination with histology of some organs with exclusion of CNS	83	40.9
4. Complete gross examination and histology limited to the placenta	37	18.3
5. Only gross examination	24	11.8
Total	203	100.0

FIGURE 1. Panel assessment on the contribution of postmortems.

POSTMORTEM TYPE	CONTRIBUTION		
	MAJOR	MINOR	NONE
1	22	4	3
2	31	4	3
3	31	10	38
4	11	4	17
5	2	3	18

Table 2 summarizes the anatomical findings in 187 available postmortems. It shows four major groups (anoxia, respiratory pathology, malformation and unaccounted) with a limited number of cases among the other diagnoses. Such a clustering can be the result of the predominance of inadequate postmortems (Table 1). This must also be the explanation for the large number of unaccounted cases (n=61) since in 54 of these the postmortem type was 3 (n=23), 4 (n=14) or 5 (n=17). The overall incidence of intrauterine growth retardation was 26%.

Table 3 lists the findings in 79 placentas available for evaluation. The most common association was that of anoxia at postmortem with

TABLE 2. Anatomical findings in 187 cases.

Anoxia	32	Birth trauma	1
GMH/IVH[a]	6	Malformation	42
Pulmonary haemorrhage	1	Immunological hydrops foetalis	1
NNEC[b]	1	Non-immunological hydrops foetalis	1
HMD/BPD[c]	21	Congenital tumor	1
Pneumonia	4	Miscellaneous	13
Meningitis	2	Unaccounted	61

a Germinal matrix haemorrhage-intraventricular haemorrhage
b Neonatal necrotizing enterocolitis
c Hyaline membrane disease/bronchopulmonary dysplasia

TABLE 3. Placenta findings (n=79).

S.U.A.[1]	4	Dysmaturity	3
Torsion	-	Abruptio (extensive)	16
True knot	1	Retroplac. haematoma	5
Funiculitis	-	Infarction (extensive)	12
U.C.[2] (other)	1	Ischaemia	3
Meconium	-	Villous fibrosis (extensive)	2
Amnion nodosum	2	Villitis	2
Chorioamnionitis	5	Tumour	-
Membranes (other)	-	Placenta (other)	23

1 Single umbilical artery
2 Umbilical cord

as additional findings a) abruptio (n=14), b) infarction (n=9), c) retroplacental haematoma (n=4), d) dysmaturity (accelerated maturation) (n=2), ischaemia (n=3) and chorioamnionitis (n=2). Malformations were related to single umbilical artery (SUA) (n=3) and amnion nodosum (n=2).

The completeness of reporting has been evaluated by matching the cases with the information available in The Medical Birth Registry of Norway and in The Central Bureau of Statistics. There was no underreporting of perinatal deaths in the present material.

A panel of experts reviewed all the deaths. The panel consisted of an obstetrician, a pediatrician, a perinatal pathologist (LAURINI, RN), a perinatal epidemiologist and a midwife. The panel classified each death into one of the following three categories:

- unavoidable deaths,
- possibly avoidable deaths, given ideal conditions, and
- possibly avoidable deaths, given present conditions.

The classification was based on information on each case collected through specially designed forms, hospital records, autopsy reports and antenatal care records. Also, oral information was available from the project coordinator of each county (pediatrician).

Some conditions were a priori considered to cause unavoidable deaths. These were: gestational age less than 26 weeks and birth weight less than 700 grams, pulmonary or renal agenesis, anencephalus, major meningomyelocele and multiple severe congenital malformations.

The causes of the perinatal deaths and the diseases or conditions of the pregnant women were classified according to the 9th Revision of the International Classification of Diseases (WHO), although more emphasis was placed on the postmortem findings. The panel restricted possible avoidable deaths to those cases where alternative actions from the health personnel might have improved the course of events. Conditions outside the control of health personnel, even though avoidable, were classified unavoidable.

The panel spent an average of 15 minutes discussing each case. All discussions were tape recorded and thus were available later for detailed registration of information and for the use of the panel in maintaining consistent criteria. The repeatability of this method of classification was evaluated by reclassification of 20 deaths. The repeatability of judgements separating unavoidable and possibly

TABLE 4. Repeatability test of the working party's decisions based on 20 deaths.

		SECOND REVIEW			
		Unavoidable cases	Possibly avoidable cases given present conditions	Possibly avoidable cases given ideal conditions	Total
FIRST REVIEW	Unavoidable cases	6	0	0	6
	Possibly avoidable cases given present conditions	0	6	1	7
	Possibly avoidable cases given ideal conditions	0	4	3	7
	Total	6	10	4	20

avoidable deaths was found to be high (Table 4).

RESULTS

The main results are shown in Figure 2. Approximately 70 per cent of the deaths were considered unavoidable, while the remainder were considered to be possibly avoidable. The majority of these (nearly 25 per cent of all deaths) were possibly avoidable, given the available facilities, which means that the deaths might have been avoided without extra resources in terms of personnel, equipment and care facilities. Figure 3 shows in which part of the perinatal period avoidable factors were located. As is apparent a considerable proportion of the avoidable deaths concerned the antepartum period. Intrauterine growth retardation was the single most common condition to be misdiagnosed or ignored during antenatal care (Table 5). At the time of birth inadequate interpretation of fetal monitoring data contributed considerably to the listed avoidable factors (Table 6).

During the neonatal period failure or inappropriate resuscitation were common factors, in addition to inadequate transfers of sick neonates (including insufficient preparation before the onset of the transfer, as well as inadequate management and treatment during the transfer (Table 7).

FIGURE 2. Main groups of perinatal deaths.

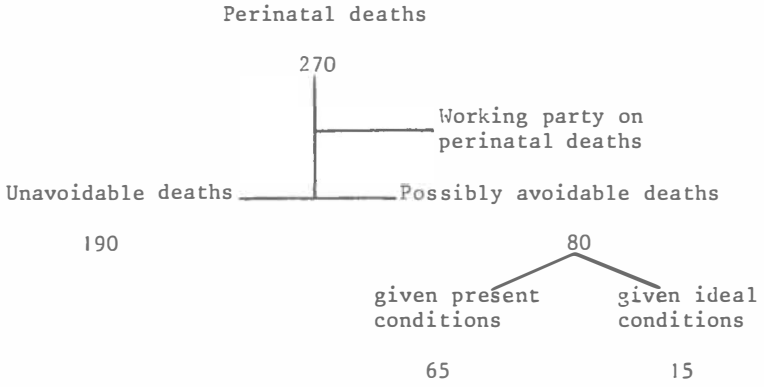

FIGURE 3. Possibly avoidable deaths given present conditions by stage of the perinatal period.

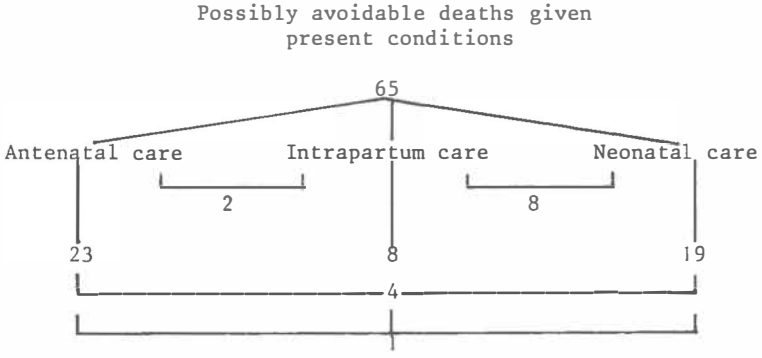

Table 8 summarizes the relationship between the main cause of death and the three categories of perinatal deaths. The resulting distribution demonstrates that approximately 30% of intrauterine deaths fall into the possibly avoidable group.

Table 9 demonstrates the relationship between pregnancy complications and intrauterine asphyxia. Moreover it underlines the importance of intrauterine growth retardation as a risk factor and emphasizes the frequent association between polyhydramnios and malformations.

TABLE 5. Possibly avoidable deaths given present conditions: possibly avoidable factors - antenatal care.

POSSIBLY AVOIDABLE FACTORS	NUMBER*
IUGR diagnosed but appropriate action not taken	12
IUGR not diagnosed despite obvious signs	4
Insufficient follow-up of high-risk pregnancies	11
Inadequate antenatal care in general	8
Other inadequate procedures	4

* Maximum 2 factors per case

TABLE 6. Possibly avoidable perinatal deaths given present conditions: possibly avoidable factors - intrapartum care.

POSSIBLY AVOIDABLE FACTORS	NUMBER*
Intrapartum monitoring carried out, but inappropriate response to abnormality	2
Intrapartum monitoring not carried out despite clear indication during labour	6
Other high risk cases in which monitoring with CTG** could have revealed hypoxia	5
Other misjudgements of the course of labour and delivery	10

* Maximum 2 factors per case
** Cardiotocography

TABLE 7. Possibly avoidable perinatal deaths given present conditions: Possibly avoidable factors - neonatal care.

POSSIBLY AVOIDABLE FACTORS	NUMBER*
Inadequate resuscitation	18
Delayed transfer or inadequate care during transport	12
Inadequate neonatal care in general	13
Inadequately performed ventilatory treatment	7

* Maximum 2 factors per case

TABLE 8. Causes of perinatal deaths by main groups.

MAIN CAUSE OF DEATH IN FETUS/INFANT	UNAVOIDABLE		POSSIBLY AVOIDABLE GIVEN PRESENT CONDITIONS		POSSIBLY AVOIDABLE GIVEN IDEAL CONDITIONS	
	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT
Intrauterine death	58	30.6	21	32.3	3	20.0
Intrauterine asphyxia/ birth asphyxia	45	23.7	7	10.8	3	20.0
Preterm	8	4.2	1	1.5	1	6.7
Malformations	51	26.8	3	4.6	1	6.7
CNS-diseases	8	4.2	6	9.2	2	13.3
Respiratory diseases	14	7.3	21	32.3	4	26.7
Other disease/ conditions	6	3.2	6	9.2	1	6.7
Total	190	100.0	65	100.0	15	100.0

TABLE 9. Main cause of fetal death by main disease/condition of the pregnant woman.

MAIN DISEASE/CONDITION OF THE PREGNANT WOMAN	MAIN CAUSE OF FETAL DEATH		
	INTRAUTERINE ASPHYXIA/ UNACCOUNTED	MALFORMATIONS	OTHERS
Pre-eclampsia/eclampsia	10	-	-
Polyhydramnios	-	6	2
Unspecified antepartum haemorrhage	3	2	-
Abruptio placentae/retro-placental haematoma	40	-	-
Other placental conditions	20	4	-
Other pregnancy related diseases	5	-	2
Multiple pregnancy	6	-	-
Labour/delivery complications	12	2	1
Other diseases not related to pregnancy	9	2	-
No maternal disease	27	12	1
Total	132(28)*	28	6

* 28 out of 82 intrauterine deaths were small for gestational age

DISCUSSION

Perinatal audit is carried out routinely in East Germany and in some states of Australia where such audits are enforced by law (SCHEIBE and VOGEL 1967, WESTERN AUSTRALIA 1978). In other countries like the United Kingdom audits have been conducted in certain areas (MAC VICAR 1980, MERSEY REPORT 1982, OWEN 1977). The results of this perinatal audit indicate that a considerable part of the perinatal deaths might be avoidable (nearly one third). Further, it should be stressed that the findings indicate that improved outcome might be obtained without necessarily any great injection of new resources in terms of personnel

and equipment. The challenge lies in improving the quality of the work within perinatal care. To arrive at such a goal the panel recommended the establishment of a regional perinatal committee in order to examine and evaluate the local organization of perinatal care, conduct perinatal audits on a routine basis and design and supervise graduate and postgraduate training programmes.

The critical function of the perinatal postmortem was shown by the fact that it represented an important contribution in the final classification of cases. A recent evaluation by clinicians demonstrated that in 26% of cases the postmortem was the only mean by which the cause of death could be determined (SHIKES et al. 1984). The same report also showed that in 42% of cases the postmortem was the sole basis for genetic counselling.
The true value of the perinatal postmortem is to be found in the percentage of cases in which it represents a significant contribution to the final understanding of the case.

The classification of postmortem into different types helped to demonstrate that partial postmortems only have a limited value. Therefore one of the recommendations from the panel was that paediatric pathology (including perinatal pathology) needs to be strengthened as a medical discipline and that the technique of perinatal postmortems should preferably be standardized. KNOWELDEN et al. (1984) have also reported that detailed paediatric necropsies revealed findings that could not have been obtained from limited postmortems.

Unaccounted intrauterine death and intrauterine asphyxia/birth asphyxia represented a significant percentage of possible avoidable deaths. Furthermore morphological changes of anoxia (CLAIREAUX 1977) were common and showed a clear relationship with placenta pathology. These are important findings since after improvements in neonatal survival following the introduction of neonatal intensive care units, reduction in asphyxial deaths, mainly hypoxic stillbirths, represents the greatest potential for further reduction in perinatal morbidity and mortality (MORRISON 1985). Moreover it is also of interest to note the significant incidence of both respiratory and CNS-diseases among the possible avoidable deaths. These causes of perinatal morbidity and mortality can benefit from earlier diagnosis of fetal distress allowing for a more optimal planning of perinatal care. In this context TAYLOR

et al. (1985) already reported strong associations between all types of neurodevelopmental disabilities and severe pregnancy induced hypertension (PIH), unclassified antepartum haemorrhage and preterm uterine activity. Recent work by NELSON and ELLENBERG (1986) further relate brain damage to prenatal rather than intrapartum risk factors.

3.2. PERINATAL MORTALITY IN A SWEDISH COUNTY 1973-1978

The perinatal mortality has fallen dramatically (50-70%) in many countries during the last decade. It is lower in Sweden than in any other country (BAKKETEIG et al. 1984). Today about 75% of the total number of deliveries in Sweden occurs in hospitals with both specialized obstetric service and neonatal units (official statistics of Sweden, 1981). A continued centralization of deliveries to this type of hospitals is recommended (SOCIALSTYRELSEN 1973, SVENSK PEDIATRIK INFÖR 1983). The justification of this recommendation can be questioned. In a series of previous studies (EKSMYR and EKLUND 1985, EKSMYR 1985a, EKSMYR 1985b) an unexpected difference in early neonatal mortality was found between two geographically defined populations with different organization of medical care. The relatively low mortality in referral districts lacking pediatric wards was not explained by underreporting or differences in perinatal risks but possibly associated with the perinatal care provided.

The aim of the present study was to examine the perinatal deaths in one referral district without direct access to neonatal care with emphasis on possible clinical events of importance for the decline in perinatal mortality, and also to evaluate the feasibility of perinatal audit as a method for analysis of time trends in perinatal mortality.

MATERIALS AND METHODS

The material is based on all births (n=8415) during the six years 1973-1978, born of mothers residing in the East Health Administration District (District East) of the county of Jönköping, Sweden. This district was one of seven referral districts originally included in one of the studies mentioned above (EKSMYR and EKLUND 1985).

Birth in District East was geographically defined, i.e. according to the mother's residence at the time of delivery and irrespective of where the birth took place. A total of 90.3% of the infants were born in Eksjö General Hospital, 8.2% in Jönköping Central Hospital and 1.5% were born outside the county. All these births were included in the analysis. The travelling time between the hospitals is 45 min by ambulance.

District East is an area composed of six rural municipalities with a total of about 115000 inhabitants. Antenatal care was provided by midwives and obstetricians, with the exception of one municipality (9.9% of the births) in which general practitioners together with midwives were responsible for the care. The attendance rate was nearly 100%. On average, each woman made 10-12 antenatal visits, including two or more examinations by a physician. Ultrasound examinations for risk pregnancies became available from 1976 onwards. Symphysis-fundal height measurements and cardiotocography during labour was available from the mid 1970s.

The low risk pregnant women resident in the six rural municipalities were scheduled to be delivered in Eksjö Hospital. Risk cases were referred to Jönköping Hospital which had a neonatal unit with round-the-clock service by paediatricians in the hospital, direct access to assisted ventilation (continuous positive airway pressure, and, if needed, ventilator), intravenous treatment, blood gas and electrolyte analysis and X-ray examinations.

Risk referrals of mothers were decided by obstetricians. Both in Eksjö and Jönköping, specialized obstetric service was available. Midwives were available continuously, and obstetricians were on 24 hours call and able to turn up within a few minutes.

Epidemiological and statistical methods. The number of births to mothers residing in District East 1973-1978, distributed by birth weight, year and place of birth, was obtained from the Swedish Medical Birth Registry. A list from the same source of all perinatal deaths was checked against locally available data. The reporting was complete. Totally, 91 perinatal deaths occurred in the years 1973-1978.

The expected perinatal deaths (PNDexp), standardized for birth weights, shown in Table 1, was obtained as follows:

$$PNDexp = \Sigma\ n_i\ p_i$$

where n_i = total births of each subgroup of birth weights (-1499, 1500-1999, 2000-2499, 2500+, unknown) and p_i = the expected PND of each subgroup, using the rates for Sweden as a whole.

TABLE 1. Number of births, perinatal deaths and early neonatal deaths by birth weight 1973-1978.

	NUMBER OF BIRTHS	PND			END		
		EXP	OBS	PAV	EXP	OBS	PAV
1973-1975							
< 1500	30	18.0	20	9	9.8	11	7
1500-1999	48	9.5	18	9	4.1	10	7
2000-2499	121	7.1	5	3	3.1	4	2
2500+	4185	20.4	17	7	7.8	6	2
no information	4	1.6	0	0	1.1	0	0
Total	4388	56.6	60	28	25.9	31	18
1976-1978							
< 1500	21	10.1	12	1	5.6	7	1
1500-1999	32	5.2	3	0	2.4	1	0
2000-2499	101	5.1	6	3	2.2	2	1
2500+	3869	14.6	10	0	6.0	4	0
no information	4	1.5	0	0	1.1	0	0
Total	4027	36.5	31	4	17.3	14	2

The expected early neonatal deaths (ENDexp) was calculated analogously. In the statistical evaluation of the observed numbers of deaths in relation to expected numbers, the Poisson distribution was applicable.

In comparing the frequencies, χ^2-test for two independent samples with Yates's correction for 2 x 2 contingency tables was used (SIEGEL 1956). When this test was inapplicable because of a too low expected number in a category, an approximate test was based on the normal approximation to the binomial distribution for comparison of two proportions was applied (COLTON 1974).

The perinatal audit method. The method of perinatal audits differs somewhat from study to study. The design of the present audit was quite similar to a recently published Norwegian audit (LARSSEN et al. 1982). The perinatal deaths in the present study were reviewed by the

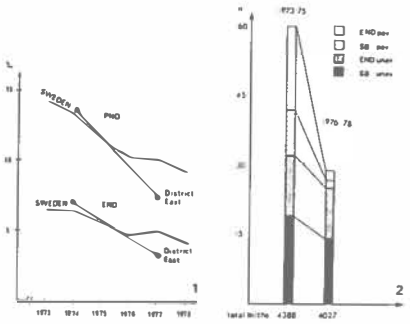

Fig. 1. Triennial perinatal mortality rates in District East in comparison with the annual perinatal mortality rates in all of Sweden 1973–1978.

Fig. 2. Triennial distribution of the perinatal deaths (n=89) classified by perinatal audit. END pav=early neonatal deaths, possibly avoidable. SB pav=stillbirths, possibly avoidable. END unav=early neonatal deaths, unavoidable. SB unav=stillbirths, unavoidable.

same Norwegian panel which consisted of an obstetrician, a midwife, a pediatrician, a perinatal epidemiologist and a perinatal pathologist. For each of the 91 cases of perinatal death in the study period, a summary of relevant antenatal, intrapartum and neonatal data was provided in beforehand to the members of the panel, together with a form with basic facts concerning the mother and the child. Postmortem reports were also available in 85.7% of the cases. One home birth and one birth during transportation were excluded from the perinatal audit. Each case was classified by the panel into one of two categories, unavoidable and possibly avoidable deaths. The classification into these main groups was supplemented by specifying one or more avoidable factors with reference to the antenatal, intrapartum and/or neonatal period. The panel considered that an avoidable factor was present when the chances of survival of a foetus or newborn might have been enhanced if some other course of action had been taken in the management of pregnant woman, her foetus or newborn baby.

Some conditions were a priori considered to cause inavoidable deaths such as gestational age less than 26 weeks and birth weight less than 700 g, pulmonary or renal agenesis, anencephalus, major meningomyelocele and multiple congenital malformations. The panel restricted possibly avoidable deaths to those cases where alternative action from the health personnel might have improved the course of events. Conditions outside the control of health personnel, even though avoidable, such

as alcohol abuse during pregnancy, repeated non-attendance at the antenatal clinic or lack of compliance were not categorized as possibly avoidable factors. The classification was based on the "state of the art" as of 1983. The panel's decisions were made without knowledge of the time of delivery.

RESULTS

Epidemiological data. The decrease in perinatal mortality in District East followed the general pattern of decline in perinatal deaths in Sweden during the study period (Fig. 1). During the first triennial period (1973-1975), the perinatal death rate in District East was 13.7‰, and in the second (1976-1978) 7.7‰. The corresponding rates in Sweden as a whole were 13.0‰ and 9.9‰. The differences in the decline in the rates were not statistically significant. Similarly, the stillbirth rate in District East decreased from 6.6 to 4.2‰ (in Sweden: from 6.9 to 5.1‰) and the early neonatal death rate from 7.1 to 3.5 per thousand live births (in Sweden: from 6.2 to 4.8‰). None of these observed rates in District East differed significantly from the expected rates (Table 1).

In all, 688 or 8.2% of the 8415 infants were transferred antenatally to a hospital with a neonatal unit. This antenatal transfer frequency did not change significantly from the first to the last period. However, considering the low birth weight (LBW<2500 g) infants separately, the rate of in utero transfers increased from 33.2% in the first to 48.1% in the second period ($p<0.01$), while the proportion of LBW infants decreased from 4.5% to 3.8%. This change in place of birth of the LBW infants is illustrated in Fig. 3. It concerned all birthweight subgroups under 2500 g uniformly, and it coincided with a marked reduction of perinatal deaths, in particular early neonatal deaths, among those LBW infants who were delivered at the Eksjö General Hospital.

The perinatal audit. The rate of perinatal deaths classified as unavoidable was almost unchanged from the first (7.3‰) to the second triennial period (6.2‰). These rates correspond to 32 cases (stillbirths: 19; early neonatal deaths: 13) in the first period and 25 cases (stillbirths: 14; early neonatal deaths: 11) in the last period. The corresponding figures for perinatal deaths classified as possibly

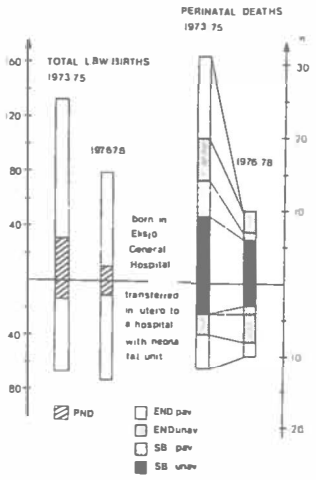

Fig. 1 Triennial changes in births and perinatal deaths of the LBW infants in District East, related to their place of birth and classified by perinatal audit. PND=perinatal deaths, END pav=early neonatal deaths, possibly avoidable, END unav =early neonatal deaths, unavoidable, SB pav =stillbirths, possibly avoidable, SB unav=stillbirths, unavoidable.

avoidable, decreased significantly ($p<0.001$) from 6.4 (28 cases) to 1.0 (4 cases) per thousand. The 28 cases of the first triennial period comprised 18 early neonatal deaths and 10 stillbirths. The 4 cases from the second period included 2 stillbirths and 2 early neonatal deaths (Fig. 2). Thus, in the first period 47% of all perinatal deaths were classified as possibly avoidable, while this proportion had fallen to less than 14% in the second period. Table 1 shows that the reduction in possibly avoidable deaths occurred in all weight groups.

The LBW births are of particular interest in an assessment of the importance of a neonatal unit located in the same hospitals as the obstetric department. Twenty-one of the above mentioned 28 cases of the first triennial period were LBW infants whose deaths (stillbirths: 5; early neonatal deaths: 16) were classified as possibly avoidable. The four cases similarly classified in the second period concerned LBW infants only. The distribution of the LBW births and perinatal deaths in maternity hospitals is shown in Fig. 3, and the possibly avoidable factors are listed in Table 2.

There was no case during the second triennial period that was classified as possibly avoidable due to lack of antenatal transfer and none of 3662 liveborn infants (73 with low birth weight) born in the general hospital during the years 1976-1978 died in the early neonatal period.

TABLE 2. Possibly avoidable factors among perinatal deaths. District East 1973-1978.

POSSIBLY AVOIDABLE FACTORS	NUMBER
Antenatal care[1]	
Small for dates diagnosed but appropriate action not taken	1
Small for dates not diagnosed despite obvious signs	2
Insufficient follow-up of high-risk pregnancies	8
Other inadequate procedures	2
Transfer before delivery possible but not carried out	6
Intrapartum care[1]	
Intrapartum monitoring carried out, but inappropriate response to abnormality	1
Intrapartum monitoring not carried out despite clear indication during labour	3
Other misjudgements of the course of labour and delivery	5
Drugs during labour with possible adverse effects on foetus/infant	2
Neonatal care[2]	
Inadequate resuscitation	10
Delayed transfer or inadequate care during transport	9
Inadequate neonatal care in general	2
Inadequately performed ventilatory treatment	12

1 Only one factor per case
2 Maximum 3 factors per case (totally 18 cases).

DISCUSSION

Several reports on perinatal audits are available from USA (The New York Academy of Medicine 1955), West Germany (SCHMIDT et al. 1973), Great Britain (Mersey Working Party on Perinatal Mortality 1982, Northern Regional Health Authority Coordinating Group 1984), Australia (The Consultative Council on Maternal and Perinatal Mortality 1981) and Norway (LARSSEN et al. 1982). The main purpose of these audits has been to identify factors influencing current perinatal mortality. In contrast, the audit presented in this study is based on material covering an earlier period. Furthermore, the appraisals in this audit were performed by assessors from another country. This had both advantages and

drawbacks. Bias due to local familiarity was avoided and the anonymity of patients and staff reduced the risk of personal considerations in more delicate matters. On the other hand, the panel's limited knowledge of the local organization and procedures decreased its ability to more detailed judgements.

The panel's decisions were based entirely upon the content of medical records. This information proved to be sufficiently complete to enable classification of all cases. Nevertheless, it is well known that such records do not give all details. When the panel was in doubt, the tendency was to classify a death as unavoidable. As documentation was more complete in the latter compared to the first triennial period, the observed decrease in avoidable deaths can hardly be ascribed to any biased documentation.

The decline in number of possibly avoidable factors between the first and the second triennial period is striking and applies to antenatal as well as intrapartum and neonatal care. With reference to the previously mentioned recommendations in Sweden to centralize all deliveries to hospitals with both obstetric and neonatal service (Socialstyrelsen 1973, Svensk Pediatrik Inför 1983), the possibly avoidable factors regarding transport seem to be of special importance in this study. In a total of 15 cases, all occurring in the first triennial period, maternal transfers before delivery or transfers of newborns, optimally performed, might have influenced the outcome. The decline of these and other avoidable factors together with epidemiological findings particularly concerning the low birth weight infants indicate that a general hospital with specialized obstetric service and without its own department of pediatrics can provide perinatal care of comparable quality, if the service is supplemented with in utero referrals of risk pregnancies and transfers of sick newborns to a central hospital with better resources.

Furthermore, perinatal audit, as defined in this study, has proved to be a useful method in elucidating possible causes underlying an observed decline in perinatal mortality. Thus perinatal audit increases the knowledge supplied by more traditional epidemiological methods.

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3.3. PERINATAL MORTALITY IN GRONINGEN

The need for a comprehensive, standardized, postmortem examination has previously been referred to (Chapter 2 (2.1) - Chapter 3 (3.1)). Here the results obtained by the routine use of a developmental post-mortem protocol as described in Chapter 2 are discussed.

RESULTS

In 1983 eighty-six complete developmental postmortems (≥ 12 weeks gestation - 4 weeks postnatal) were carried out at the Department of Pathology, University of Groningen. In Table 10 the study population is summarized. Sixty-five postmortems were from the University Hospital (Groningen), while the other twenty postmortems were referrals from pathologists from peripheral hospitals while in only one instance it was required by a general practitioner.

Despite the recognition of the high prevelance and associated increase in perinatal morbidity and mortality (BUTLER and ALBERMAN 1969) intrauterine growth retardation (IUGR) is not considered in reports on causes of perinatal deaths (NAKAMURA et al. 1982). The percentage (22%) of IUGR in this series was similar to that in the norwegian material (26%) (see 3.1).

The two types of IUGR have been discussed in Chapter 2. The most common association (7 out of 10 cases) of the asymmetrical type was with hyaline membrane disease (HMD), bronchopulmonary dysplasia (BPD), lung immaturity and/or hypoplasia, germinal matrix-intraventricular haemorrhage (GMH-IVH) and anoxia. The most frequent relationship (4 out of 9 cases) of the symmetrical type was with congenital malformations and chromosomal abnormalities.

Late fetal deaths are important because further reduction in perinatal

TABLE 10. Data on 86 developmental postmortem.

< 17 wks. GA	11	intrauterine deaths*
17 < 28 wks. GA	21	
late fetal deaths	16	
preterm	20	liveborns
< 1500	17	
BW > 1500 < 2500	5	
> 2500	16	
I.U.G.R.(A)	10	
I.U.G.R.(S)	9	

GA : Gestational age
BW : Birthweight
I.U.G.R.(A): Intrauterine growth retardation (asymmetrical)
I.U.G.R.(S): Intrauterine growth retardation (symmetrical)
* : Includes 13 elective terminations.

mortality might result from the elimination of many of these intrauterine deaths (Table 11).

It is of interest to underline the presence of IUGR associated to lung hypoplasia and oligohydramnion sequence. MANNING et al. (1981) have postulated that one mechanism for oligohydramnios in growth-retarded human fetuses may be a decreased production of fetal urine.

Table 12 and 13 list the findings in the 86 postmortems and 47 placentas available for examination.

The incidence of anoxia might seem low but it must be stressed that this diagnosis was used only when morphological changes were present (CLAIREAUX 1977). In the absence of identifiable anatomical lesions (including anoxia) the case was classified as unaccounted. In the view of this author this is preferable to using anoxia as a basket for otherwise unexplained cases even in the absence of morphological changes result of perinatal hypoxia.

The percentage of unaccounted cases varies from series to series (as discussed by NAKAMURA et al. 1982) and is very dependent on the quality of the developmental postmortem (see 3.1) as well as on the definition of anoxia. The percentage (6%) observed in this study represents unaccounted on a morphological basis.

A number of authors have recently stressed the need for a more detailed birthweight-specific analysis of perinatal mortality rates

TABLE II. Late fetal deaths (n=16)*

POSTMORTEM DIAGNOSIS	BIRTHWEIGHT(g)	COMMENTS
Siamese twins	2980	Elective termination at 34 weeks gestation Diprosopus+NTD[1]+CHD[2]
Anencephalic	730	Elective termination at 28 weeks gestation
Hydrocephalus	2250	Elective termination at 34 weeks gestation
Thanatophoric dysplasia	1520	Elective termination at 31 weeks gestation
Anencephalic	785	Twin II, lung hypoplasia
Anoxia	890	Abruptio (clinical)
Anoxia	1775	Retroplacental haematoma
Anoxia	2950	CHD
GMH-IVH[3]	1550	3 previous consecutive intra-uterine deaths at 25, 37 and 27 weeks gestation
Lung hypoplasia-IUGR(S)[4]	675	Retroplacental haematoma Previous child dysmature (24 weeks gestation)
Lung hypoplasia-IUGR(A)[5]	700	Placenta infarction (extensive) Oligohydramnion sequence
Ectopic (ovarian) pregnancy	2225	-
Hydrops foetalis non-immunol.	3780	Lung hypoplasia
Unaccounted	1170	-
Unaccounted	2310	Torsion umbilical cord?

* Late fetal deaths: fetal deaths with a gestational age of 28 weeks or more per 1000 total births

1 Neural tube defect
2 Congenital heart defect
3 Germinal matrix haemorrhage-intraventricular haemorrhage
4 Intrauterine growth retardation (symmetrical)
5 Idem (asymmetrical)

TABLE 12. Anatomical findings in 86 developmental postmortems.

Anoxia	4	Meningitis	1
GMH/IVH[1]	12	Infection (other)	1
CNS Haem. (other)	2	Birth trauma	1
PVL[2]	4	Iatrogenic	8
Pulmonary haemorrhage	4	Congenital heart defect	18
Haemorrhage (other)	-	Malformation (other)	18
NNEC[3]	3	Chromosomal	5
HMD[4]	10	Metabolic	-
BPD[5]	6	Immunological hydrops foetalis	1
Lung hypoplasia/ immaturity	13	Non-immunological hydrops foetalis	2
Congenital pneumonia	5	Congenital tumor	2
Pneumonia (other)	1	Miscellaneous	19
		Unaccounted	5

1 Germinal matrix haemorrhage-intraventricular haemorrhage
2 Periventricular leucomalacia
3 Neonatal necrotizing enterocolitis
4 Hyaline membrane disease
5 Bronchopulmonary disease

TABLE 13. Placenta findings (n=47).

SUA[1]	3	Dysmaturity	5
Torsion	1	Abruptio (extensive)	2
True knot	-	Retroplac. haematoma	6
Funiculitis	4	Infarction (extensive)	4
UC[2] (other)	4	Ischaemia	1
Meconium	-	Villous fibrosis (extens.)	-
Amnion nodosum	2	Villitis	-
Chorioamnionitis	6	Tumour	-
Membranes (other)	-	Miscellaneous	11
		No findings	12

1 Single umbilical artery
2 Umbilical cord

TABLE 14. Neonatal deaths (< 1500 g) (n=17).

MAIN PATHOLOGY		ASSOCIATED FINDINGS
HMD	7	GMH-IVH(5) PVL(3) iatrogenic(3) lung hypoplasia-IUGR(2)
GMH-IVH	2	Lung hypoplasia-IUGR(2)
NNEC	2	Pulmonary haemorrhage
BPD	1	IUGR
Lung hypoplasia	1	IUGR
Trisomy	2	IUGR(1)
Unaccounted	2	Immaturity(1)
		IUGR(1)

For meaning of abbreviations see Tables 11, 12 and 13.

(CHALMERS 1979, MACFARLANE 1981). Table 14 shows the main pathology and associated findings in the group of very low birthweight (< 1500 g). The significant percentages of both hyaline membrane disease and intraventricular haemorrhage for this birthweight group despite improved perinatal care is in agreement with the experience of others (BARSON et al. 1984). As with regard to respiratory pathology it is important to note the high incidence of lung hypoplasia and/or immaturity since this has a great influence in neonatal prognosis.

Table 15 summarizes the findings in neonatal deaths with a birthweight between 1500 and 2500 g and over 2500 g.

The observed pathology (Table 15) is of significance since BAKKETEIG et al. (1978) demonstrated that the association between available perinatal services and perinatal survival was stronger for births with average to above average weights than for low weights infants. Therefore they emphasized that when monitoring the possible effects of improvements in care one should not merely concentrate on the outcome among low weight infants.

The data on congenital heart defects (Table 15, birhtweight > 2500 g) reflects the active involvement of the University Hospital (Groningen) in the field of management of congenital heart disease.

Table 13 illustrates the placenta findings in this series of

TABLE 15. Neonatal deaths.

BIRTHWEIGHT (g)	MAIN PATHOLOGY		ASSOCIATED FINDINGS
> 1500 < 2500 (n=5)	Meningitis	1	IUGR
	BPD	1	IUGR
	HMD-BPD	2	GMH-IVH(2), PVL(1), NNEC(1) Iatrogenic(1)
	Holopros-encephaly	1	Trisomy 13, IUGR
> 2500 (n=16)	CHD	12	-
	Anoxia	1	Congenital pneumonia + chorioamnionitis + grey matter necrosis (brain) SUA + CHD
	HMD	1	-
	Birth trauma	1	Breech + tentorium rupture + posterior fossa haemorrhage
	Unaccounted	1	Asphyxia + hepatomegaly

For meaning of abbreviations see Tables 11, 12 and 13.

perinatal postmortems. We have already referred to the placenta pathology in embryonic and fetal deaths (Chapter 4). The most frequent placenta changes in perinatal deaths were those of inflammation and vascular pathology that showed a clear relationship to anoxia.
Placenta dysmaturity was diagnosed when placenta maturation did not correspond with the gestational age of the case. It was associated with congenital anomalies (n=2), anoxia (n=1, accelerated maturation), hydrops foetalis, non-immunological (n=1) and unaccounted (n=1). In cases when there is a retarded maturation (relative immaturity) the changes are similar to those seen in diabetes (Chapter 6). The significant incidence of dysmaturity indicates that not only placenta pathology but also placenta development plays a part in perinatal disease.
As with regard to villitis it is the experience of this author that the presence of characteristic morphological changes are less frequent in placentas from perinatal deaths than from liveborns.
Finally there is still a large percentage of placentas with changes difficult to interprete that underline the need for further study of

this organ in the context of the materno-feto-placental unit.

Nevertheless the findings in this series demonstrate the essential contribution of the placenta to perinatal disease, although it was available for examination in only 55% of cases. Therefore this author (LAURINI 1986) recommends that the following placentas are to be routinely sent for morphological examination: a) from all fetal deaths, b) from all liveborn with a birthweight < 2500 g or > 4000 g, c) in case of admission to a neonatal intensive care unit, d) in case of a positive obstetrical history (fever, pregnancy induced hypertension, diabetes etc.). As with regard to (c) it is recommended that neonatal intensive care units require (in their protocol) that the placenta is sent together with the neonate in cases of referral from other institutions immediately or soon after birth.

GENERAL DISCUSSION

In this chapter it has been established the essential role of the developmental postmortem in the assessment of perinatal mortality. Furthermore the critical function of the developmental postmortem has been also emphasized in relation to the use of the perinatal audit as an instrument to identify factors that influence perinatal survival.

The data obtained from the postmortems performed in the Department of Pathology (University of Groningen) understandingly reflect the situation of the University Hospital as a referral centre for high-risk pregnancy, neonatal intensive care and services in the field of medical genetics.

Nevertheless, Groningen forms part of the EUROCAT-REGION (CORNEL et al. 1986) and one can therefore assess the representativeness of the postmortem findings from Groningen in terms of the expected number of postmortems for the EUROCAT-region. In 1983 the expected number of postmortems in the period from 17 weeks gestation to 4 weeks after birth, for the EUROCAT-region, was 132 as calculated from CORNEL et al. (1986) and Table 1 - Chapter 1. Seventy-five out of the 86 postmortems carried out in Groningen in 1983 correspond to the period above mentioned and represent 57% of the expected number of postmortems. In this relation and bearing in mind that 21 of the 86 postmortems were referrals from peripheral pathologists, the findings in this selected

group of perinatal postmortems can be considered indicative of the expected range of perinatal pathology to be anticipated in the EUROCAT-region.

Still without a regional organization of a perinatal audit along the lines of the two Scandinavian studies it will not be possible to properly analyse the causes of perinatal deaths. This should be arranged on the basis of a representative population that combines both central (high-risk obstetrics, neonatal intensive care, medical genetics) as well as peripheral centres. In this relation the already established EUROCAT-region (CORNEL et al. 1986) represents a possible choice.

Although there has been a sharp decline in perinatal mortality and morbidity through the years, it still represents a significant health problem for the different countries (HOOGENDOORN 1986). Still one should not use this parameter to judge perinatal services or compare one country with another before a careful evaluation of the different factors that influence perinatal mortality figures. Perinatal mortality is very dependent on the birthweight distribution, ethnic composition, technological development and the delivery of health care services within individual countries (HOFFMAN et al. 1984). Therefore one should assess ethnical groups separately, evaluate perinatal mortality and morbidity in birthweight-specific groups with the help of a comprehensive postmortem and establish a regional perinatal audit to inquire into possible avoidable factors that will allow for the change of management necessary to further reduce both perinatal mortality and morbidity.

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CHAPTER 4: PATHOLOGY OF EARLY PREGNANCY

INTRODUCTION

It has been suggested (Chapter 1 - Fig. 1) that the period between the end of embryogenesis and beginning of extrauterine viability represents a well defined developmental stage with physiopathological characteristics of its own. The diagnosis of fetal abnormalities has evolved rapidly over recent years mainly as result of the introduction of fetal imaging techniques, amniocentesis, fetoscopy and the chorion villous biopsy. Moreover the establishment of genetic counselling together with todays approach to family planning further highlight the importance of the pathology of early pregnancy. Besides the detection of fetal defects may nowadays lead not only to modification in obstetrical management but also to prenatal treatment (ADZICK 1985). This has resulted in an increasing pressure for a detailed morphological examination of embryonic and fetal deaths, which is usually lacking (HALL 1979).
Such a pathological assessment is part of a clinicopathological correlation that will allow for a better understanding of both the etiology and pathogenesis of fetal changes. This, in itself, is an important step in the establishment of possible ways of therapy and/or prevention. Therefore the anatomical study of embryonic and fetal deaths, regardless whether the result of spontaneous or elective termination of pregnancy, must not be limited to the pathology of malformations, chromosomal anomalies and genetical disease, but include all forms of embryonic and fetal injury (i.e. hypoxia).

The pathology of early pregnancy comprises the study of embryonic (till 8 weeks) and fetal deaths (from 8 up to 24 completed weeks of gestation).

4.1. PATHOLOGY OF FETAL DEATHS

MATERIAL AND METHODS

In order to ascertain the pathology of fetal deaths we reviewed

95 consecutive fetal deaths, that comprised all cases between 12 and 24 completed weeks of gestation for 1982-1984. There were 69 cases (72.6%) of spontaneous deaths while 26 cases (27.4%) represented elective terminations (for maternal disease n=4, because of antenatally diagnosed fetal abnormality n=21, for social reasons n=1). A complete postmortem was carried out in all cases (see chapter 2), postmortem X-ray examination was done in 93 cases (97.9%), while the placenta was examined in 78 (82.1%) of the cases. In addition obstetrical data were available in 76 (80%) of the cases and showed that there were 4 recurrent and 11 habitual abortions in the group of spontaneous deaths as opposed to 2 cases of habitual abortion among the elective terminations.

RESULTS

As expected the terminations based on antenatal diagnosis resulted in a significantly higher incidence of X-ray lesions. Table 3 summarizes the radiographic findings for both groups.

The examination of the placenta showed abnormal findings in 42 of the 55 placentas available from spontaneous fetal deaths, in contrast to only 5 of the 23 available from elective terminations (Table 2). Table 4 lists the observed placenta lesions in both groups.

TABLE 2. Abnormal findings at postmortem (95 cases).

	Spontaneous abortions n=69 (72.6%)	Elective terminations n=26 (27.4%)
X-Ray examination (n=93)	9	15
Placenta examination (n=78)	42	5
Complete postmortem (n=95)	20	24*

* only additional findings

The most important finding was the high incidence of chorioamnionitis that was commonly associated with fetal hypoxia-ischaemia. Placenta haemorrhage in the fetal period, in similarity to the abruptio placenta of the third trimester, represents a haemorrhagic separation and its

TABLE 3. Radiography findings.

	Spontaneous abortions (n=69)	Elective terminations (n=26)
X-Ray available	67	26
Craniospinal anomalies	2	4
Skeletal dysplasia	-	1
Other skeletal anomalies	3	3
Pathological calcifications	1	2
Lung hypoplasia	1	-
Other soft tissue abnormalities	1	4
Hydrocephaly	1	-
Microcephaly	-	1

TABLE 4. Placenta findings.

	Spontaneous abortions (n=69)	Elective terminations (n=26)
Placenta available	55	23
SUA_1	3	-
Torsion $(U.C.)_2$	1	-
Velamentous insertion (U.C.)	2	1
Amnion nodosum	-	1
Chorioamnionitis	12	-
Placenta haemorrhage	10	-
Ischaemia	3	1
Intervillitis	4	-
Placenta dysmaturity	2	-
Miscellaneous	5	2

1 single umbilical artery
2 umbilical cord

importance lies in that it can be a hallmark of uteroplacental arterial disease. The occurrence of placental dysmaturity (accelerated or retarded maturation for gestational age) indicates that placenta development also contributes to the pathology of the fetal period. The miscellaneous consisted of changes compatible with a rubella infection (n=2), presence of stromal trophoblastic inclusion without accompanying chromosomal abnormality (n=1), nucleated red blood in fetal capillaries in the absence of infection or Rhesus antagonism (n=1) and unexplained extensive iron deposit in the basal membrane of villi (n=3).

Table 5 summarizes the prenatal diagnosis and postmortem findings in elective terminations. A complete postmortem examination allowed for the recognition of 24 significant abnormalities in addition to the prenatal diagnosis (Table 2). Table 5 clearly demonstrates that even in cases of elective termination following antenatal diagnosis there is an essential contribution from a complete postmortem. Moreover it allows for the recognition of hitherto unreported changes as the abnormalities of the nephrogenic zone seen with trisomies. These consisted of focal absence of nephrogenic zone alternating with areas of hyperplasia, mainly of tubular structures.
In addition the presence of significant pathology in social terminations (n=1) further strengthens the need for a postmortem examination to be carried out in all cases of fetal death.
Major abnormalities were present in 20 cases (29%) of spontaneous fetal deaths (Table 6). This high incidence of significant pathology indicates that these fetal deaths must also be routinely examined and that one should not limit oneself to the elective terminations.
A complete postmortem of these fetal deaths, as in the case of elective terminations, can help to further expand our knowledge on fetal changes. In this context it is of interest to point out that the 2 cases of intraspinal tumour and renal agenesis were seen in sibs.

It has been emphasized already that the anatomical examination of fetal deaths must also pay special attention to the hypoxic lesions. These lesions were present in the majority of cases but exhibited a marked variability. Hypoxic-ischaemic changes affected all organs with a distinct predilection for the brain, heart, kidney, liver and adrenals; occasionally also the gastrointestinal tract and spleen.

TABLE 5. Postmortem findings in elective terminations (26 cases).

PRENATAL DIAGNOSIS		ASSOCIATED ABNORMALITIES
Trisomy	7	Abnormal nephrogenic zone (3) Abnormal ovaries (1)
Medical termination	4	Brain haemorrhage (1)
Rubella	2	Abnormal early corticogenesis (1)
Anencephalic	3	Variable degree of spinal cord dysplasia (3)
Spina bifida	1	Cerebellar aplasia, myelodysplasia Brain haemorrhage
Hydrocephalus	1	Dandy-Walker Brain haemorrhage
Abnormal fetal movements	1	Aplasia external granular layer in the cerebellum
Chromosomal translocation, cystic hygroma	1	Myocardial calcification
Cystic hygroma	1	(Isolated finding in male fetus)
Urinary tract obstruction	1	Urethral valve, Megacystic, Renal dysplasia
Abdominal cyst (renal?)	1	Congenital brain tumor, Proximal duodenal dilatation to distal duodenal atresia
Ectopia cordis + CHD	1	Brain haemorrhage
Skeletal dysplasia	1	Dyssegmental dwarfism
Social	1	Diastomatomyelia

In this context cases of elective termination of pregnancy represent a good model for acute intrauterine hypoxia following prostaglandin induction. Table 7 summarizes the brain findings in eight mid-trimester medical terminations, six of which are included in Table 5. It can be seen that the brain lesions were predominantly haemorrhagic in character which is in agreement with alterations in cerebral blood flow resulting from an acute episode of fetal hypoxia.
These findings were likened with those of a group of spontaneous fetal

TABLE 6. Postmortem findings in spontaneous fetal deaths (69 cases).

Congenital pneumonia	5	Abnormal early corticogenesis	1
Brain haemorrhage	2	Renal dysplasia (unilateral)	1
H.F. non-immunological brain haemorrhage*	1	Renal + lung hypoplasia	1
Congenital spinal tumor + renal agenesis	2	Bilateral cleft lip	1
Prune belly	2	Pulmonary arteriovenous fistula	1
Hydrocephalus Multiple anomalies	1	Congenital lymphangectasia	1
Hydrocephalus Hydromyelia	1		

* hydrops foetalis non-immunological

TABLE 7. Intrauterine brain lesions in elective termination of pregnancy.

GESTATION	BRAIN LESION	PRENATAL DIAGNOSIS
16 wks	Cortical haemorrhage	Spina bifida
17 wks	GMH[1] + IVH[2] SAH[3]	Obstructive uropathy
18 wks	GMH - IVH parenchymal extension	Perfusion treatment for malignant melanoma
18 wks	laminar necrosis	Turner
18 wks	GMH	Skeletal, Dysplasia
19 wks	SAH GMH parenchymal haemorrhage	Trisomy 21
19 wks	CPH[4]	Hydrocephalus Dandy Walker
25 wks	GMH	Ectopia cordis Congenital heart defect

1 Germinal Matrix Haemorrhage; 2 Intraventricular Haemorrhage
3 Subarachnoidal Haemorrhage ; 4 Choroid Plexus Haemorrhage

TABLE 8. Intrauterine brain lesions in spontaneous fetal deaths.

GESTATION	BRAIN LESION	OTHER ABNORMALITIES	PLACENTA
15 wks	IVH[1]	Hydrocephalus Hydromyelia	Normal
16 wks	GMH[2] - IVH	Congenital pneumonia	Chorioamnionitis
19 wks	IVH	Hydrops foetalis Non-immunological Lung hypoplasia	Hydropic
19 wks	GMH - IVH	Congenital pneumonia	Chorioamnionitis
20 wks	GMH - IVH	-	Ischaemia
22 wks	GMH - IVH	Myocardial ischaemia	Chorioamnionitis
25 wks	GMH - IVH	I.U.G.R.[3]	-
26 wks	PVL[4] + Haemorrhage	I.U.G.R.	RPH[5] Infarction 10%
26 wks	GMH - IVH	I.U.G.R.	Infarction 40%
28 wks	Falx-tentorium haemorrhage Gliosis	PIH[6]	Infarction 40%
30 wks	Falx-tentorium haemorrhage Gliosis	PIH	Infarction 40%

1 Intraventricular Haemorrhage
2 Germinal Matrix Haemorrhage
3 Intrauterine Growth Retardation
4 Periventricular Leucomalacia
5 Retroplacental Haematoma
6 Pregnancy Induced Hypertension

deaths seven of which are included in table 6 (up to 25 weeks) (Table 8). In the latter there was also a preponderance of haemorrhagic lesions but non-haemorrhagic changes as periventricular leucomalacia (PVL) and gliosis were also present, the latter indicating to a more chronic fetal hypoxia.

DISCUSSION

The use of X-ray examination as part of the developmental postmortem is mandatory today (Chapter 2). Moreover the case of dyssegmental dwarfism reinforces the fact that radiographic appearance is crucial to the differential diagnosis of skeletal dysplasias (SILLENCE et al. 1978, MAROTEAUX et al. 1976). In this family 2 previous sibs had been diagnosed at postmortem as thanatophoric dysplasias. On review both these cases proved to be dyssegmental dwarfs. The importance of this differential diagnosis lies in the fact that thanatophoric dysplasia is sporadic while dyssegmental dwarfism can show an autosomal recessive inheritance as in our case (HANDMAKER 1977). SILLENCE et al. (1978) discuss the evidence that negate the previously held concept that thanatophoric dysplasia might be inherited as an autosomal recessive trait. GRAHAM et al. (1984) further stress the essential role of fetal postmortem radiography and recommend the use of xeroradiography to enhance visualization of soft tissues. In our experience the use of Faxitron (see Chapter 2) allows for comparable results.

Although there is an increasing number of studies on the morphological findings in embryos and fetuses, reports of early placenta pathology are less common. Nevertheless our findings are in agreement with those described by ORNOY et al. (1976 and 1981). They also stress the importance of infection in the mid-trimester and state that nearly 100% of their cases showed inflammatory changes (deciduitis, chorioamnionitis, villitis) (ORNOY et al. 1976). The difference in the incidence of inflammatory lesions with our study is due to: a) we used the term chorioamnionitis only when both the chorion and the amnion showed inflammatory changes, b) we did not register deciduitis since we found it difficult to differentiate it from the degenerative changes commonly seen in the decidua of embryonic and fetal deaths. In our series 5 of the 12 cases of chorioamnionitis also showed a congenital pneumonia (Table 6). In a later report of ORNOY et al. (1981) the percentage of inflammatory lesions was significantly lower. In this study they also refer to the occurrence of ischaemia in placentas of first and second trimester. RUSHTON (1984a) also reports a significant incidence of chorioamnionitis, retroplacental haemorrhage and uteroplacental ischaemia associated with fresh and macerated fetuses.

In summary the observed placental lesions clearly demonstrate the critical role played by the placenta in the context of the pathology of spontaneous fetal deaths. Moreover it further underlines the need for a routine examination of this organ.

There are few anatomic studies on fetal deaths (mid-trimester) and they focus on congenital anomalies, chromosomal abnormalities and genetic disease (WINGATE 1968, HAXTON and BELL 1983). Little attention is payed to the associated abnormalities and the common findings of fetal hypoxia and related placental pathology. This study demonstrates that a comprehensive postmortem examination can contribute significantly even in cases following antenatal diagnosis. The associated abnormalities here described help to better understand the individual case as well as to increase our knowledge of fetal development and disease which allows for better patient counselling.

Nevertheless the predominant pathology was that of fetal hypoxia-ischaemia. In view of the fact that neurodevelopmental handicap represents an established parameter for the evaluation of perinatal outcome hypoxic-ischaemic intrauterine brain damage was used to highlighten the importance of acute and chronic fetal hypoxia. Further analysis showed some interesting and significant associations. There was an obvious relationship between placenta pathology and brain damage (Table 8). Placenta ischaemia, mainly when associated with extensive infarction and pregnancy induced hypertension, can give rise to a more chronic fetal hypoxia that results in changes of gliosis. On the other hand chorioamnionitis seemed to be related to haemorrhagic lesions as seen with elective terminations. I have interpreted this as the result of fetal hypoxia secondary to the release of prostaglandins (PG) that has been reported with chorioamnionitis (BEJAR et al. 1981).

All taken into consideration this study demonstrates the need for a routine comprehensive postmortem examination for all fetal deaths including social terminations. This is of vital importance since, for example, the recurrence risk of a neural tube defect will depend on whether it represents an isolated defect or is associated with trisomy 13, triploidy, autosomal recessive inheritance (i.e. Meckel's syndrome) or an amniotic band syndrome. Indeed a recent report by RUTLEDGE et al. (1986) demonstrates that 52 malformations were diagnosed prenatally by ultrasound in 42 patients but 90 additional malformations were not. Furthermore nine sonographically diagnosed abnormalities

were not confirmed at postmortem.

In conclusion the most important aspects of fetal pathology are the congenital anomalies, chromosomal abnormalities, genetic disease and hypoxic-ischaemic pathology. The latter is significantly related to inflammatory and/or ischaemic pathology of the placenta.

4.2. PATHOLOGY OF EMBRYONIC DEATHS

The pathology of the first 8 weeks of pregnancy is predominantly related to chromosomal abnormalities usually in combination with a variable degree of embryo growth-disorganization while specific defect(s) represent only a minority of cases (KALOUSEK and POLAND 1984). Still normal embryos with normal karyotypes represent approximately 15% of the total (see Chapter 1) so that other form(s) of pathology must be considered.

The available classifications of spontaneously aborted products of conception (MALL and MEYER 1921, FUJIKURA et al. 1966, HERTIG 1968) are mainly descriptive and without relationship to routine histopathology services. A more recent classification, based primarily on the placenta findings (RUSHTON 1984a) does not require detailed anatomical dissection or fetal histology and is aimed at routine histopathology services without specialized equipment and staff.
His group 1 (blighted ova - mean ovulation age 9.4 weeks) and group 2 (macerated fetuses - mean ovulation age 14.1 weeks) show however mainly changes secondary to intrauterine death while group 3 (fresh fetuses - mean ovulation age 18.6 weeks) shows lesions similar to those already discussed under fetal deaths.
Therefore if pathology is to enhance our knowledge on the pathophysiology of embryonic deaths we need a systematic approach to the morphological assessment of the embryonic period that covers the embryo-gestational sac, placenta and maternal structures (decidua) to include the utero-placental circulation.

METHODS

The guidelines for the morphological assessment of embryonic deaths has been discussed in Chapter 2. The working classification of first

trimester abortions (Appendix 3) resulted from a retrospective study of the available material and other data in 486 specimens. In view of the limitations of the retrospective analysis the morphological changes were only used to define groups that showed the most common findings. Therefore group 1 comprises cases with only degenerative changes after intrauterine death. Group 2 includes haemorrhagic lesions defined as an extensive dissecting decidual haemorrhage with or without extension into intervillous space. Group 3 shows hydropic changes without trophoblast proliferation. Group 4 is defined by the presence of molar lesions with variable degree of trophoblast proliferation. Group 5 groups the ischaemic changes and group 6 is reserved for abnormal findings that cannot be classified into any of the other groups. The latter group includes changes of intervillitis (extensive polymorphonuclear infiltration of perivillous fibrin) and of extensive polymorphonuclear (PMN) infiltration around foci of feto-maternal haemorrhage. Placenta ischaemia was diagnosed in the presence of "accelerated maturation" (RUSHTON 1984b) with or without infarctions.

RESULTS

The majority of cases corresponded to group 1. Although a correlation with cytogenetics was not possible the association of simple hydropic abortion (group 3) with blighted ova or growth-disorganized embryos suggests that many of these hydropic abortions are the result of chromosomal abnormalities. In several of these blighted ova the presence of nucleated red blood cells in fetal capillaries indicated the previous existence of a non-viable embryo that had undergone resorption.

Placenta haemorrhage was a frequent finding usually represented by a retroplacental bleeding that, due to the normally thin early decidua basalis, had ruptured into the intervillous space. This lesion was commonly seen with normal embryos.

A variable degree of placenta ischaemia was present in most specimens. Nevertheless it was easy to recognize a group (group 5) with extensive ischaemic changes usually combined with multiple foci of infarction. Moreover if the site of placenta implantation was present (trophoblast invasion of decidua) the decidual portions of the spiral arteries showed the same lesions as described for the myometrial portion in

hypertensive pregnancy (ROBERTSON et al. 1967). These cases with placenta ischaemia were also usually associated with normally developed embryos. This group included several cases of repeated early pregnancy loss that after 6, 8 or even 12 abortions succeeded in a normal pregnancy. In addition this group included four consecutive spontaneous abortions with extensive infarction in one woman with phlebothrombosis (leg) and presence of lupus anticoagulant (LA) without lupus disease.

Among the miscellaneous (group 6) it is of interest to comment on the lesion of extensive PMN infiltration around foci of feto-maternal haemorrhage seen in two cases. In one such case the mother (G7PO-AB Rh+) presented with a threatened abortion on week 10 of her eighth pregnancy. At that time the patient had an elevated anti-A titre (1/8000) and was started on prednisolone (10 mg per day). There was a reduction in the anti-A titre (1/512) but the patient aborted at 22 weeks gestation. The fetus was normally developed and the placenta showed the above mentioned changes. In addition fetal red blood cells collected from this placenta were covered by anti-A antibodies. In her tenth pregnancy the patient was started on prednisolone (40 mg per day) as soon as the pregnancy test became positive. This treatment was continued until delivery, at 29 weeks, following a Caesarean section for fetal distress. This baby is now a healthy infant.

The changes of intervillositis were usually seen together with placenta ischaemia. Still in few cases the intervillositis was the only lesion present.

In this series of embryonic deaths the lesions of chorioamnionitis and villitis were present only once and in separate cases.

DISCUSSION

The results of this retrospective study demonstrate that there is more to embryonic pathology than anomalies, chromosomal aberrations or genetic disease. The findings of placenta haemorrhage associated to normal embryos suggest that the events reminiscent of abruptio placenta and retroplacental haemorrhage also take place in early pregnancy. Another important form of pathology seen with normal embryos was that of placenta ischaemia. When the placenta site was available for examination the arterial changes were similar to those originally

described at a later stage in pregnancy (ROBERTSON 1967). The changes described by ROBERTSON et al. (1975) refer to the result of an abnormal placentation about the 14th to 16th week of gestation secondary to an insufficient second wave of endovascular trophoblastic migration that reduces the maternal blood flow supply to the conceptus.

In normal conditions the first wave of endovascular trophoblastic migration occurs in the decidual portion of spiral arteries before the 12th week of gestation (BROSENS 1967).

Therefore this author suggests that early ischaemic pathology of the gestational sac or placenta is also the result of a deficient utero-placental circulation secondary to pathology of the decidual portion of spiral arteries. Similar findings have been reported by NADJI and SOMMERS (1973).

Severe placenta ischaemia with extensive infarction was also related to lupus anticoagulant without lupus disease. The adverse effects of lupus anticoagulant on pregnancies, its association with thrombosis as well as the possible absence of lupus disease have been previously reported (ANONYMOUS EDITORIAL, LANCET 1984)

It is suggested that lupus anticoagulant is tested in patients with recurrent habitual abortion and repeated extensive infarction of the placenta (gestational sac) without clinical signs of lupus since prednisone treatment may lead to successful pregnancies (LUBBE et al. 1983).

The occurrence of isolated intervillositis and of an inflammatory response around foci of abnormal mixture of maternal and fetal blood suggest a possible immunological mechanism. The findings on the case here reported including the successful treatment with prednisolone strengthen this hypothesis.

GENERAL DISCUSSION

In the past scant attention was paid to the pathology of early pregnancy. The pathologist usually limited himself to the confirmation of pregnancy and eventual trophoblastic disease. Nowadays the developments referred to at the introduction of this chapter clearly establish a need for a comprehensive morphological examination of both embryonic and fetal deaths.

The findings reported in this chapter demonstrate the wide range of significant pathology present in the different types of early pregnancy loss. Furthermore it highlights the essential role played by hypoxia-ischaemia. The latter must be considered as an equally important factor together with anomalies, chromosomal abnormalities and genetic disease, in the assessment and classification of embryonic and fetal deaths.

Although this review of fetal trimester specimens has the limitations of a retrospective study it does provide with useful information necessary for the planning of a systematized morphological examination of embryonic deaths that will help understand the problems of early pregnancy loss.

Embryonic and fetal deaths represent a common, geographically widespread event and its morphological examination cannot depend on the services provided by the usually few trained developmental pathologists available. Furthermore one must also consider the ongoing development of antenatal diagnosis outside teaching centres. For these reasons it is realistic to foresee that pathologists attached to many general hospitals will be obliged to provide a service in developmental pathology. In this context the pathologist has much to offer if he performs a detailed morphological evaluation following established standard procedures. One must bear in mind that it is still impossible to screen all patients for potentially detectable abnormalities and high risk groups have to be defined. Pathology plays here a central role not least because it represents a cost-effective approach that allows for an earlier start of a more comprehensive antenatal care of future pregnancies. A rewarding antenatal diagnosis and surveillance can only result of a close collaboration between clinicians and pathologists.

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CHAPTER 5: DEVELOPMENTAL NEUROPATHOLOGY

INTRODUCTION

Developmental neuropathology plays a major role in the context of perinatal morbidity and mortality. In general terms one can classify the possible lesions into those that result from hypoxia-ischaemia, infection, an established developmental anomaly or a metabolic defect.

Despite recent advances in perinatal care the work of McCORMICK (1985) demonstrated that the incidence of major neurodevelopmental handicaps, among the low birth weight infants, has remained unchanged over the past decade. Furthermore recent observations by TAYLOR et al. (1985) indicate that neurodevelopmental disability may, in many cases, be the result of pregnancy pathology.

Perinatal brain damage of haemorrhagic and/or ischaemic origen represents, today, the most commonly recognized brain lesion in perinatal postmortems. The advent of ultrasound and computed tomography have further emphasized the frequency and importance of these lesions.

All this strongly underlines the need for a detailed neuropathological examination as part of the perinatal postmortem. The importance of such a comprehensive morphological assessment of the development and patholology of the perinatal central nervous system derives also from the need to carefully evaluate the result of active neonatal intensive care in the very low birth weight population.

The introduction of modern ultrasound allows not only for the assessment of fetal anatomy but also of fetal function including fetal behavioural states (TUCK 1986). This has represented a dramatic turn in the approach to the pathology of early pregnancy since now the anatomical findings at postmortem can be correlated to both the intrauterine structural as well as functional status. As a matter of fact, at present, ultrasound and postmortem pathology represent complementary methods that potentiate each other in the evaluation of human fetal developmental pathology.

5.1. ABNORMAL MOTOR BEHAVIOUR IN ANENCEPHALIC FETUSES

SUMMARY

In eight anencephalic fetuses ultrasound observations of movement patterns were made and correlated with the morphological findings at postmortem. In all fetuses the movements were qualitatively abnormal: they were forceful, jerky in character and of large amplitude. In some of the most defective cases classification of the movements was hardly possible as they showed little similarity to those observed in normal fetuses. In these cases movements tended to occur in burst-pause patterns in contrast to being scattered over the record. Excessive activity occurred also only in the more defective cases. In fetuses with no evident cervical cord present isolated arm movements were observed. Fetal lung hypoplasia occurred as early as 16 weeks and in a fetus which showed both hiccups and breathing movements. It is concluded that with a severely defective fetal central nervous system, already in the first half of pregnancy movement patterns are abnormal. This abnormality mainly concerns the quality of the different movements. Secondly, movements can occur despite severe reduction in the amount and alteration in the organisation of the fetal central nervous system.

INTRODUCTION

Before birth development of the motor function of the nervous system can be studied by ultrasound observations of fetal movements. From normal fetuses it is known that from early gestation onwards movements are specific and well recognizable and closely resemble those observed in preterm and term infants (DE VRIES et al. 1982). This makes them of diagnostic value in the assessment of the integrity of the central nervous system.

With this in mind we studied movement patterns in anencephalic fetuses and compared these data with the morphological findings after termination of pregnancy. Research in these severely defective fetuses seemed of interest as it might provide answers to the following questions.

1. Are there qualitative and quantitative differences of the various categories of movements as compared to normal fetuses?
2. What is the relationship between the severity of the morphological abnormality and the degree of abnormal motor behaviour?
3. What is the minimal neural structure required for the generation of fetal movements?

PATIENTS AND METHODS

Real-time ultrasound observations were made in seven pregnant women in whom the diagnosis of anencephaly had been made. One woman was carrying a twin pregnancy of whom both fetuses were anencephalic (cases no. 2 and 3). One patient had type-1 diabetes, the others were healthy. The duration of gestation (calculated from the first day of the last menstrual period) at the time of diagnosis as well as at the time of fetal movement recording varied from 16 to 35 weeks (Table 1).

Continuous real-time ultrasound observations of 30 (n=3) or 60 (n=4) min duration were made with the women lying in a semi-recumbent position. The transducer of a linear array real-time scanner (Searle 2300 or Aloka 256) was held so that the fetus was visualized in the mid- or parasagittal section. The scanning images of each session were recorded on videotape together with the output of a digital clock in order to locate each observed event in a precise moment of time.

Analyses of fetal motility was carried out during replay of the video recordings. The incidence and duration of different movements were marked on an event recorder (Hewlett-Packard, 7754A), using hand-held push buttons. Movements were classified using a classification system which has previously been published (DE VRIES et al. 1982). The quantity of individual movements was compared to that of normal fetuses, matched for gestational age (DE VRIES et al. 1985); for this purpose the results of 30 min recordings were extrapolated to 1 h.

In all cases pregnancy was terminated within 3 days of the fetal movement recording, using a prostaglandin E_2 derivate infusion (Nalador). A complete postmortem (including X-ray examination) was carried out in seven of the anencephalic fetuses. In six the central nervous system was removed in toto, fixed in 10% formalin, serially blocked and embedded in paraffin. In one case (no.4) the central

nervous system was removed together with the corresponding skeletal structures (cranium-vertebral column) in order to avoid damage of the severely malformed nervous system. After formalin fixation the head was decalcified and serially blocked while the vertebral column and spinal cord together were serially blocked and paraffin embedded. Serial 5 µm sections were stained with hematoxylin-eosin, Perl's (iron) and Masson trichrome. In case no. 4 all tissue blocks were serially sections in order to examine histologically the neural axis in its total length.

The various segments of the nervous system were categorized as being near-normal ("normal"), dysplastic or aplastic. "Normal" is defined by the presence of white and grey matter structures corresponding to that found in normal fetuses with comparable gestational age. Dysplasia indicates anomalies in the arrangement and the amount of white and/or grey matter, whereas with aplasia no white and/or grey matter structures corresponding to brain or spinal cord could be identified: in the latter only fetal lepto-meninges and glial tissue were sometimes present, together with dorsal ganglions.

As part of a complete perinatal postmortem lung weights were obtained. The data of wet weight of the lungs were compared to those of an extended series of normal fetuses, taking into consideration gestational age and birthweight (POTTER and CRAIG 1976). In view of the limited data available on normal fetal organ weights in early gestation, lung hypoplasia was only thought to be present if lung weight was extremely low (< 50% of normal weight).

All morphological examinations were carried out by one of the authors (R.L.) without having any knowledge of the observed fetal movement patterns.

RESULTS

Morphology. The morphological findings of the central nervous system of the seven fetuses in which a complete postmortem was carried out, are summarized in Figure 1 and in Table 1. Figure 1 shows the level below which a more or less normal or dysplastic nervous system could be identified. In Table 1 details of the individual fetuses are given. In case no. 8, recorded at 35 weeks, no permission was given for a perinatal postmortem. However, the antenatal heart rate pattern of

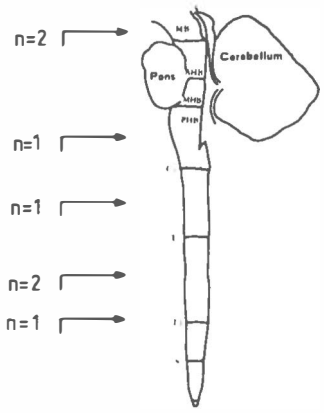


Fig. 1. Level below which a "normal" or dysplastic central nervous system could be identified in the seven anencephalic fetuses in which a complete postmortem was carried out.

TABLE 1. Morphological findings of the central nervous system in seven anencephalic fetuses in which a complete postmortem was carried out.

Case no.	Duration of gestation (in weeks)	Forebrain and midbrain	Hindbrain			Spinal cord		
			Pons	Cerebellum	Medulla	Cervical	Thoracic	Lumbosacral
1	16	-	-	-	+	++	++	++
2	16	-	++	+	++	++	++	++
3	16	-	++	+	++	++	++	++
4	17	-	-	-	-	-	+	+
5	17	-	-	-	-	-	++	++
6	19	-	-	-	-	-	++	++
7	33	-	-	-	-	-	+	+
8	35							

++ "normal" finding, + dysplasia, - aplasia

this fetus revealed a normocardia, normal long-term heart rate variation and the presence of accelerations. According to two reports in the literature comparing anencephalic fetal heart rate patterns with the degree of the morphological abnormality, the heart rate pattern of

this fetus (case no. 8) suggests the presence of both pons and medulla (DE HAAN et al. 1971, TERAO et al. 1984). Apart from this case an "intact" hindbrain, with pons and medulla and dysplastic cerebellum, were only found in the twin fetuses. A dysplastic medulla was found in one other case. In the remaining four fetuses only parts of the spinal cord were present; in three of these the cord was aplastic in the cervical region. This was associated with a cervical rachischisis.

Movement patterns. In all fetuses there was a poorly differentiated movement pattern and on average not more than 4-5 specific movements could be identified (Table 2). In some of the most defective cases classification of the movements was hardly possible as they showed little similarity to those observed in normal fetuses and newborn infants. Generalized movements were present in alle fetuses; isolated arm movements in all but one, and startles and isolated leg movements in all but two of the fetuses. Breathing was observed on three occasions. Hiccups, which occur frequently during the first half of pregnancy in normal fetuses, were observed in only one of the six fetuses recorded before 20 weeks.

TABLE 2. Occurrence of specific movement patterns in eight anencephalic fetuses.

Movement category	Case no. (duration of gestation (wks) in brackets)							
	1(16)	2(16)	3(16)	4(17)	5(17)	6(19)	7(33)	8(35)
General movements	+	+	+	+	+	+	+	+
Startle		+	+	+	+		+	+
Isolated arm movements	+	+	+	+		+	+	+
Isolated leg movements		+	+	+		+	+	+
Breathing movements	+			+				+
Hiccup				+				
Hand/face contact		+		+		+		
Head rotation	+							
Head retroflexion								
Head anteflexion								
Jaw opening								
Sucking + swallowing								
Stretch								
Yawn								

The clearest abnormality concerned the quality of the individual movements and especially that of the general movements. In all fetuses these movements were forceful, jerky in character and of large amplitude and caused large positional shifts in the uterus. They started abruptly, and during the movement the same force and amplitude continued until the movement suddenly stopped. This is in contrast to the fluent appearance and the waxing and waning of these movements in normal fetuses. Although there were large interfetal differences the execution of these movements was highly consistent within the individual fetus. Isolated arm and leg movements were also jerky in character, as was breathing in the 33 week old fetus (case no. 7).

The temporal patterning of the general movements was abnormal in five of the eight cases. In the twin fetuses (cases no. 2 and 3) and in case no. 8 these movements were scattered throughout the record, whereas in the others they tended to occur in burst-pause patterns (Fig. 2).

The incidence of generalized movements (expressed as percentage of recording time) correspond in three cases to the median values as found in normal fetuses of similar gestational ages (cases no. 2, 3 and 8; incidence 10.5-12.5%). In the remaining five cases, the incidence was above average and in three of these excessive activity was found, with an incidence exceeding the normal range (cases no. 1, 4 and 6; incidence 21-28%). In the records made before 20 weeks the incidence of startles always exceeded the median values found in normal fetuses.

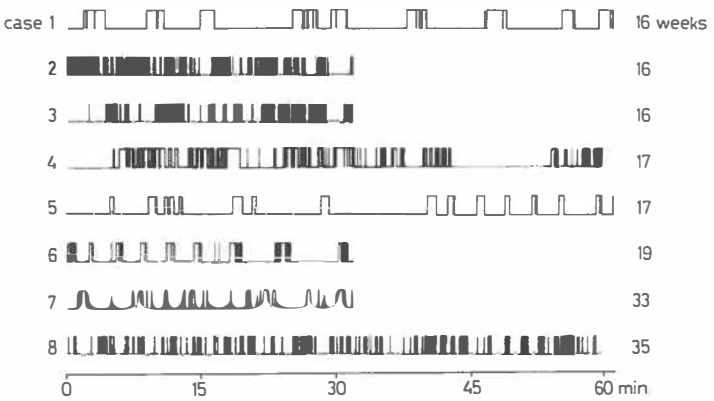


Fig. 2. General movement pattern in the eight anencephalic fetuses. Each upward deflection indicates an observed movement.

The incidence of most other specific movement patterns was usually low: breathing 0.2-2% of recording time; isolated arm movements 7-34 per hour; isolated leg movements 6-22 per hour; hand-face contact 1-2 per hour. Hiccups were present in only one fetus (case no. 4) and appeared excessive (266 hiccups, 18.3% of recording time; Fig. 3).

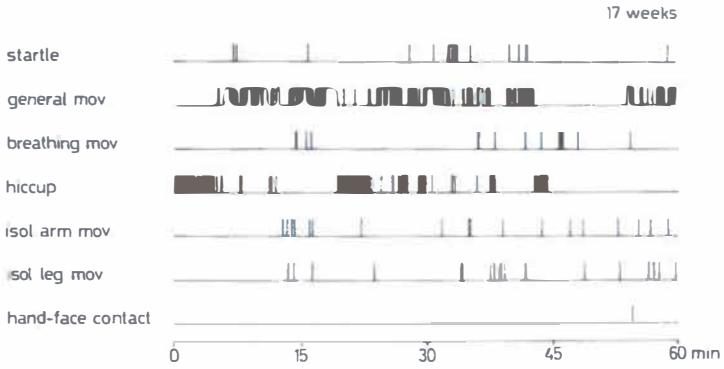


Fig. 3. Compiled actogram of a 1-h observation of case no. 4. In this severely defective anencephalic fetus spinal cord was only present at the lower thoracic and lumbosacral level.

Relation between morphology and movement patterns. The occurrence of burst-pause patterns and the presence of excessive activity (general movements occupying more than 20% of recording time) were clearly related to the degree of morphological abnormality (Table 3).

TABLE 3. Relation between central nervous system morphology and the occurrence of burst-pause movement patterns and of excessive motor activity (general movements > 20% of recording time).

Hindbrain structures	n	Burst-pause movement pattern	Excessive activity
Present	3	-	-
Absent	5	5	3

Assuming that case no. 8, with a normal antenatal fetal heart rate pattern, had an "intact" pons and medulla, all three cases with an intact hindbrain had a normal distribution and incidence of general movements. In the more defective cases movements tended to occur in burst-pause patterns and in three excessive activity was present.

As mentioned before, classification of the movements was especially difficult in the more defective cases as these movements showed only little similarity to those observed in normal fetuses.

From Figure 1 and Table 1 it can be seen that in cases no. 4, 6 and 7 the cervical cord was absent. However, in all three cases, isolated arm movements were present while in one (case no. 4) breathing and hiccups were also observed. A compiled actogram of this case is shown in Figure 3. In this particular case (the most defective one of the series) a detailed morphological examination was carried out (see Methods). At the cervical region only fetal meninges, scarce glial tissue and dorsal ganglia were present with no spinal cord at all. A hypoplastic and dysplastic cord was only found at the lower thoracic level (Fig. 4A); at this level a few motor neurones with a haphazard orientation were present (Fig. 4B,C). Few other neurones were seen in connection with prominent dorsal root ganglia and can, therefore, not be classified as motor neurones. Groups of motor neurones were identified only in the lumbosacral segment. These data indicate how little central nervous system structure is required for the generation of (abnormal) movements.

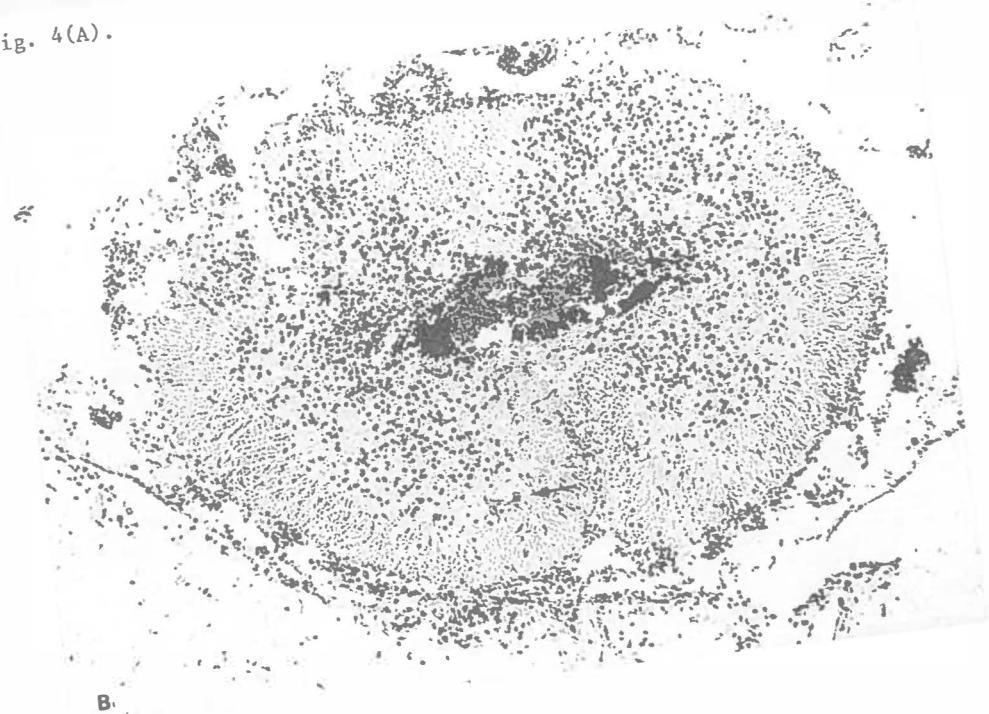
In three cases lung hypoplasia was present (lung weight < 50% of normal weight). Two of these were delivered before 20 weeks and in one (case no. 4) both hiccups (18% of recording time) and breathing (2% of recording time) had been present.

DISCUSSION

In normal fetuses movements emerge early, are from the beginning specific and well recognizable and closely resemble those observed after birth (DE VRIES et al. 1982). This early emergence of specific movements, in the earliest stages of development of the fetal nervous system, is puzzling. The present study indicates that already in the first half of gestation a normal fetal nervous system is necessary for a normal appearance and patterning of movements and, secondly, that only very little central nervous system structure is necessary for (abnormal) movements to occur at all. The data show that even without any evident cervical cord isolated arm movements can be found. As it is not realistic to presume that movements occur without motor

A

Fig. 4(A).



B

Fig. 4(B)

(For text see page 89).

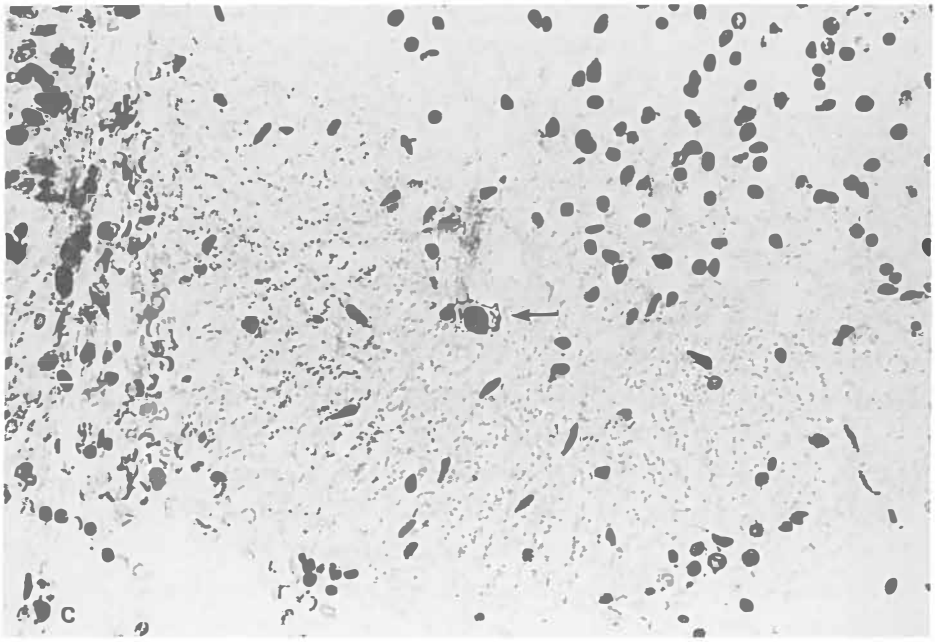


Fig. 4(C).

Fig. 4. Case no. 4 (A) Overview of the thoracic segment showing a hypoplastic spinal cord with mild dilatation of the ependymal canal and prominent dorsal ganglion; hematoxylin-eosin. (B) Dysplastic spinal cord shown in (A). Note reduction of white matter, dilated ependymal canal with hemorrhage, and grey matter formed mainly by glial cells with an occasional neurone (arrow) but no groups of motor neurones present. 60x, hematoxylin-eosin. (C) Higher magnification of (B) showing isolated motor neurone (arrow). 350x, hematoxylin-eosin.

neurones being present, an ectopic localization of these neurones, either inside or outside the central nervous system, seems to be the most likely explanation. So in early fetal life movements need only a few motor neurones to emerge, but an intact, although poorly developed, nervous system to be executed normally. Even in fetuses younger than 20 weeks, it could be demonstrated that the degree of abnormal motor behaviour was related to the severity of the morphological abnormality. This held for both the quality and patterning of movements as well as for the occurrence of excessive fetal activity. Also for fetal heart rate patterns a relationship with the degree of the morphological abnormality has been established (DE HAAN et al. 1971, TERAO et al. 1974).

The most striking difference to the normal fetus was seen in the quality of the movements. The abrupt and forceful character of the movements was also acknowledged by some of the women. Two of them described this as "I thought it was going to be a football player". The hectic movement pattern of anencephalic fetuses was already noted by PREYER in 1885. The fact that with a severly defective nervous system qualitative movement changes are more striking than the overall quantity of movements agrees with findings in less defective preterm infants and compromised fetuses in which qualitative differences are also more clear than quantitative ones (BEKEDAM et al. 1985, PRECHTL and NOLTE 1984). It seems, therefore, that the quality of fetal movements is an important indicator of the integrity of the nervous system.

Excessive fetal activity was, on the other hand, observed in three of the eight cases. This is in contrast to the findings of RAYBURN and BARR (1982), who reported that the incidence of movements of anencephalic fetuses, assessed subjectively by the pregnant women, was always within normal limits. Their study, however, included only four anencephalic fetuses and their main interest concerned the prognostic significance of fetal inactivity.

Fetal breathing movements appear to have a profound influence on lung growth, as in animal experiments spinal cord transection results in fetal lung hypoplasia (LIGGINS et al. 1981, WIGGLESWORTH and DESAI 1979). Lung hypoplasia is common in anencephalic fetuses and it has been suggested that this is due to the absence of breathing movements, whereby the presence of "normal" breathing indicates normal

lung development (DORNAN et al. 1984). The present study shows however, that in these fetuses - just as in cases with severe oligohydramnios (FOX and MOESSINGER 1985) - lung hypoplasia can occur in the presence of both hiccups and "normal" breathing. It furthermore shows, that lung hypoplasia can be found as early as 16 weeks of gestation.

It is concluded that in anencephalic fetuses: (1) movement patterns are qualitatively and quantitatively abnormal; (2) the degree of abnormal motor behaviour is related to the severity of the morphological abnormality; (3) movements can occur in the presence of only a few motor neurones; and (4) lung hypoplasia may be found as early as 16 weeks and in the presence of both hiccups and breathing.

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5.2. CORRELATION BETWEEN POSTMORTEM ANATOMICAL AND ULTRASOUND FINDINGS IN BRAINS OF NEWBORN INFANTS

INTRODUCTION

Despite recent advances in perinatal care, germinal matrix haemorrhage-intraventricular haemorrhage (GMH-IVH) and related pathology remain a common perinatal problem. Although haemorrhages are readily diagnosed by ultrasound there is still uncertainty about the identification of hypoxic-ischaemic brain damage (HIBD).
The latter has received increasing attention since the assocation of parenchymal involvement with GMH-IVH seems to account for the high incidence of neurodevelopmental handicaps in these infants.
In order to attempt a better assessment of ultrasound findings as well as to improve our knowledge on parenchymatous lesions associated to GMH-IVH we compared sonographic findings with results of postmortem studies of brains in a series of perinatal deaths.

PATIENTS AND METHODS

Patients. Sonographic assessment was carried out in 20 cases consisting of 2 still births (twins), 17 neonatal and 1 post-neonatal death. The clinical data of these patients is summarized in Table 1. In 18 cases ultrasonography was performed within 24 hours of death. For the remaining 2 cases ultrasound scans made prior to death (3 and 60 hrs) were available.

Ultrasound. Ultrasound (US) scans were performed with a Philips SDR sector scanner equipped with a 5 MHz transducer. Scans were carried out through the anterior fontanelle in both coronal and sagittal planes (see 5.3). Areas of increased density were considered as representative of haemorrhages while decreased density was used as an indication of ischaemic brain damage. Widespread non-delineated increased density associated with decreased ventricular size were the parameters used for the diagnosis of generalized cerebral edema. The presence of increased echodensity in the cisterna magna, basal cisternas and/or interhemispheric tissue and/or silvian fissure, was considered to represent subarachnoidal haemorrhage. The sonographic and postmortem findings were first assessed independently, then compared and the sonographic diagnoses reviewed.

Autopsy. A complete perinatal postmortem including X-ray examination was carried out within 48 hrs in all cases (Chapter 2). The central nervous system (CNS) was examined and processed following the guidelines established in Chapter 2.

Most brains were sectioned following the usual coronal slices. In a few cases, at postmortem, the fontanelle site was marked on the brain in situ. These brains were further sectioned following the sonographic planes, one hemisphere coronal and the other sagittal.

The main pathology, other than brain haemorrhage and/or hypoxic-ischaemic lesions, is summarized in Table 1.

The brain findings were tabulated following our classification (LAURINI in press). This classification intergrates morphological diagnoses with the ultrasound grading as described by RUMACK and JOHNSON (1984). Only lesions identified on gross examination were considered for

TABLE 1. Clinical data and postmortem findings.

	Sex	Gest. Age	Birth-weight	Centile	Delivery	Apgar score 1'	Apgar score 5'	R.D.S.[1]	Age at death(d)	Main pathology other than CNS abnormalities
I	M	28	890	2.3	C.S.	4	8	H.M.D.[2]	23	BPD[6], obliterative bronchiolitis, obstructive pneumonia, growth retardation
II	M	26	1100	75	V.	2	4	H.M.D.	2	H.M.D., AIPE[7]
III	M	28	1060	10-25	V.	8	10	H.M.D.	2	H.M.D.
IV	M	26	600	5-10	V.	0	0	---	SB[5]	Lung hypoplasia, IUGR(A)[8]
V	F	31	960	5	C.S.	8	10	H.M.D.	3	H.M.D., bilateral pneumothorax, lung hypoplasia, IUGR(A)
VI	M	32	1120	2.3	C.S.	2	2	H.M.D.	2	H.M.D., BPD, IUGR(microencephalic)
VII	F	29	1270	50	V.	8	9	H.M.D.	3	H.M.D., myocardial ischaemia
VIII	M	40	4200	95-97.7	V.	8	8	No	4	CHD[9], macrosomia
IX	F	25	680	<2.3	V.Br.	4	6	H.M.D.	1(4h)	Immaturity, IUGR(S), nodular blastema
X	M	28	765	<2.3	C.S.	7	9	H.M.D.	2	H.M.D., IUGR(A)
XI	F	27	930	50	V.	2	4	H.M.D.	1(2h)	H.M.D., pneumothorax
XII	F	26	860	50	V.	0	1	H.M.D.	1(2h)	H.M.D., lung hypoplasia, pneumothorax
XIII	M	41	4200	90-95	C.S.	2	3	M.A.S.[3]	3	H.M.D., pulmonary alveolar haemorrhage, amniotic fluid aspiration
XIV	M	26	760	10-25	V.	0	0	---	SB	Immaturity
XV	F	32	1290	10-25	C.S.	1	1	H.M.D.	3	H.M.D., BPD, NNEC[10]
XVI	M	29	1305	25-50	C.S.	5	7	T.T.N.[4]	17	NNEC, pulmonary alveolar haemorrhage
XVII	M	40	3930	75-90	V.	u.	u.	Pneumonia	9	CHD, acute necrotizing tracheitis
XVIII	M	40	3190	25	V.Br.	9	10	Hypoventilation	3	Tentorium rupture
XIX	M	29	800	<2.3	C.S.	1	7	H.M.D.	180	BDP, renal papillae necrosis, growth retardation
XX	M	37	4020	>97.7	C.S.	1	6	No	3	CHD, changes as in diabetes, macrosomia

1 Respiratory distress syndrome; 2 Hyaline membrane disease; 3 Meconium aspiration syndrome; 4 Transient tachypnoea neonate; 5 Still birth; 6 Bronchopulmonary dysplasia; 7 Acute interstitial emphysema; 8 Intrauterine growth retardation, (S) symmetrical, (A) asymmetrical; 9 Congenital heart defect; 10 Neonatal necrotizing enterocolitis.

comparison with ultrasound findings. Histological examination was carried out on standard blocks (Appendix 14) as well as on blocks from each lesion. Tissue samples were embedded in paraffin and sections were stained with hematoxylin-eosin, PAS and Perl's stain for iron.

SCHEMA 1. Correlation between postmortem findings and ultrasound diagnosis.

POSTMORTEM FINDINGS \ ULTRASOUND DIAGNOSIS	H	SAH	ITH	CYST	CE	PVL	PH	PVL+H	IV	III	II	I	0
H	1												
SAH		1											4
ITH			1										1
CYST													2
CE					2								
PVL					1	1							
PH													
PVL+H													
IV					1	1			1				
III													
II									1	1			
I										1			2
0		1									3	4	5
	H	SAH	ITH	CYST	CE	PVL	PH	PVL+H	IV	III	II	I	0

H: Hydrocephalus
SAH: Subarachnoidal haemorrhage
ITH: Infratentorial haemorrhage
CE: Cerebral oedema
PVL: Periventricular leucomalacia
PVL+H: PVL + haemorrhage
PH: Parenchymal haemorrhage
Grade I, II, III, IV
0: No lesion

RESULTS

Comparison between postmortem and US findings can be readily done in as much as both are based on a morphological assessment. We found no

real advantage in sectioning the brain following sonographic planes and usual coronal sectioning allowed for optimal correlation.

There was a good overall correlation between sonographic and postmortem diagnoses as with regard to the number of haemorrhagic lesions. The grade I not identified on US measured 5 mm in diameter while two diagnoses of grade I haemorrhages were not confirmed by postmortem (schema 1). Furthermore a grade 3 haemorrhage was not diagnosed on sonographic examination. In two occasions there was a disagreement in the grading due to difficulty in the recognition of ventricular dilatation and haemorrhagic extension into brain tissue. Furthermore a case of haemorrhage into periventricular leukomalacia (PVL) and one of a parenchymal haemorrhage were classified as grade IV on US.
Lesions related to ischaemic brain damage, as PVL and PVL plus haemorrhage, showed a less promising correlation. To a certain degree this applied also for cerebral edema (CE) and, more so, to subarachnoidal haemorrhages (SAH). The two cysts not seen by ultrasound measured 6 and 10 mm in major diameter. The smaller cyst was immediately anterior to the left side ventricle, in an area of PVL. The larger cyst, left parietal, was subcortically situated and was probably secondary to a parenchymal haemorrhage without continuity with the GMH-IVH present in the same case.

A second look, with knowledge of the postmortem findings, allowed for a better interpretation of the sonographic findings as with regard the diagnosis and grading of haemorrhagic lesions (schema 2). Still, disagreements related to the size of the lesion, haemorrhagic extension into white substance, infratentorial localization and, mainly, the identification of areas of ischaemic brain damage remained (schema 2).

Histological examination helped to distinguish between haemorrhagic extension into white matter with or without the PVL. Furthermore Perl's stain occasionally demonstrated the presence of iron in macrophages seen at the site of recent PVL and/or haemorrhage indicating repeated bleedings. In addition multiple cortical petechial bleedings were seen, mainly in the cerebellar cortex although occasionally also in the brain cortex. These small haemorrhages sometimes ruptured into subpial or subarachnoidal space.

SCHEMA 2. Correlation between postmortem findings and reviewed ultrasound diagnosis.

POSTMORTEM FINDINGS	REVIEWED ULTRASOUND DIAGNOSIS									
	H	SAH	ITH	CYST	CE	PVL	PH	III	II	I
0										
I										1
II									4	5
III							3	1	1	
IV PVL+H						1	1			
PH					1	1	5			
PVL					1	1				
CE					2					
CYST										
ITH			1							
SAH		2								
H	1									
0										
I										1
II									4	5
III							3	1	1	
IV PVL+H						1	1			
PH					1	1	5			
PVL					1	1				
CE					2					
CYST										
ITH			1							
SAH		2								
H	1									

For meaning of abbreviations see Schema 1.

DISCUSSION

Our group of cases showed the already well established relationship between haemorrhage and ischaemic brain lesions with prematurity, respiratory distress syndrome and other forms of perinatal hypoxia (ANONYMOUS EDITORIAL, LANCET 1984).

In agreement with other publications our findings confirm that ultrasound is an accurate method for the identification and grading of germinal matrix and intraventricular haemorrhage (ROMACK and JOHNSON 1984). Still, as reported by others, small haemorrhages measuring 5 mm or less, were difficult to diagnose (PAPE et al.

1983). Furthermore marked congestion of the germinal matrix or choroid plexus in the immature infant could be misinterpreted as representing a small haemorrhage. Also haemorrhagic extension from a GMH into the white matter whether in the presence or absence of PVL was not easily recognized by US.

In addition a parenchymal haemorrhage associated with a subarachnoidal haemorrhage in a full-term neonate with congenital heart disease proved difficult to classify by US. This lesion was morphologically consistent with those previously reported by BURGER et al. (1978) as result of venous thrombosis in patients with dehydration, infection and cardiac disease.

Although 12 of our cases were premature neonates younger than 30 weeks gestation, there was no indication of choroid plexus haemorrhage in this group. This is in agreement with the findings of DONAT et al. (1978).

Correlation between computed tomography (CT) and US diagnosis with findings at postmortem have been previously reported (PAPE et al. 1983). Their results, as ours also confirm the reliability of US or CT scans for the diagnosis of haemorrhagic lesions. Nevertheless comparison between both CT and US diagnoses with autopsy findings showed poor correlation with HIBD other than haemorrhage (FLODMARK et al. 1980, see 5.3).

This is an all important point in as much as parenchymal involvement seems to be the most significant factor in the later development of neurodevelopmental sequelae (DE VRIES et al. 1985). Moreover it is of immediate prognostic value since there is published evidence that the prognosis of GMH-IVH is good whereas it is frequently very poor with PVL (LEVINE et al. 1983). Our findings also clearly illustrate the difficulties around the sonographic diagnosis of parenchymal lesions, mainly in the form of PVL with or without associated haemorrhage as described by ARMSTRONG et al. (1974).

As far as subarachnoidal and infratentorial haemorrhages are concerned our experience is similar to that previously published in as much as these lesions were difficult to diagnose on scans (FLODMARK et al. 1980).

In conclusion we found a good correlation between postmortem and US findings of germinal matrix and intraventricular haemorrhages. In our experience sonographic diagnosis of parenchymal involvement, mainly that of non-haemorrhagic PVL, remained poor. The same applied for subarachnoidal and infratentorial lesions.

Furthermore a morphological assessment not only added significantly to the identification and grading of lesions but also demonstrated the widespread brain pathology that is associated with GMH-IVH and non haemorrhagic ischaemic-hypoxic brain damage.

5.3. RELIABILITY OF SONOGRAPHY IN NON-HAEMORRHAGIC PERIVENTRICULAR LEUCOMALACIA

ABSTRACT

Sonographic pictures of the brain of 19 newborn infants who died at a mean age of 4.2 days after birth (range 1-23 days) were examined independantly by five experienced sonographers. In all infants information on postmortem brain pathology was available. Diagnoses made by the sonographers based on the sonographic pictures were compared with the gross postmortem findings. The results of the study show that except for one infant with a subarachnoid haemorrhage all cerebral haemorrhages were diagnosed accurately by all sonographers. Non-haemorrhagic periventricular leucomalacia (PVL), however, was missed on sonography in two of the three cases. Surprisingly, PVL was diagnosed on sonography in one (8%) to six (50%) of twelve infants in which postmortem examination of the brain revealed no PVL. It is concluded that non-haemorrhagic PVL cannot be diagnosed accurately using sonography in the first days of life, if a 5 MHz transducer is used.

INTRODUCTION

Hypoperfusion of the brain, especially in preterm infants, may lead to hypoxic-ischaemic brain damage (DE REUCK et al. 1972, ARMSTRONG and NORMAN 1974). If hypoxic-ischaemic brain damage results in white matter necrosis, periventricular leucomalacia (PVL) will occur. PVL may be complicated by secondary haemorrhage. One to three weeks after the ischaemic event, cystic lesions may develop in both PVL with and without secondary haemorrhages (BAERTS and MERADJI 1985, SCHELLINGER et al. 1984). Several studies (SCHELLINGER et al. 1984, HILL et al. 1982, LEVENE et al. 1983) have shown that sonography is a reliable technique

to detect PVL with secondary haemorrhage and/or cystic lesions. Recently NWAESEI et al. (1984) reported that sonography also is a reliable tool to detect non-haemorrhagic PVL. Their conclusion is based on the findings in infants with a postnatal age ranging from 3 to 36 weeks. Since the neurodevelopmental prognosis for PVL is bad (BOWERMAN et al. 1984, SAUERBREI 1984), it is important for the clinician to be able to detect PVL early. The accuracy of sonography in detecting PVL in an early stage is, however, still doubtful. The present study was undertaken to investigate the accuracy of sonographic diagnosis of non-haemorrhagic PVL in newborn infants in the first days of life.

PATIENTS AND METHODS

The study involved 19 infants who died in the neonatal period. In all infants sonography and postmortem examination of the brain were performed. Gestational age of the infants was 31.1 $\pm$ 5.4 weeks (mean $\pm$ SD), birthweight was 1.74 $\pm$ 1.32 kg. The infants died at a mean age of 4.2 days (range 1-23 days).

There were no significant differences between infants with and without PVL as regards birthweight, gestational age, Apgar scores, or time of death. Infants with PVL died at a mean age of 5 days (range 1-23 days).

Sonography was performed with a Philips SDR 2000 sector scanner, equipped with a 5 MHz transducer. The brain was scanned through the anterior fontanelle, and pictures were taken at standard coronal and sagittal planes. In all infants sonograms made within 24 hours of death were available. All sonograms were examined by a panel of five experienced sonographers from different neonatal intensive care units in The Netherlands. Examiners were informed about gestational age, birthweight, delivery, and Apgar scores, but they were unaware of the clinical course of the infants.

According to BOWERMAN et al. (1984) PVL was suspected when there were noncircumscribed echodense areas in the periventricular regions i.e. lateral to the frontal horns of the lateral ventricles and lateral to the trigone of the lateral ventricles. Increased intense echo reflections in these areas were considered to represent secondary haemorrhage. Echodense areas present in only one plane were considered to be artefacts.

Localized areas of increased echodensity were considered to represent

haemorrhage (RUMACK and JOHNSON 1984).

Postmortem examination of the brain was carried out according to LAURINI (in press). The brains were sectioned in the coronal plane; standard blocks, including areas prone to PVL (BANKER and LARROCHE 1962), were embedded in paraffin and were subsequently stained with haematoxylin-eosin, PAS and Perl's stain for iron. On gross examination, small white spots in the periventricular region were diagnosed as PVL. On histological examination the diagnosis PVL was made when changes as described by BANKER and LARROCHE (1962) were present. These changes are: coagulation necrosis, microglial reaction, reactive astrocytosis, prominent macrophages and, finally, cavitation. More extensive infarction of the periventricular white matter, with or without secondary haemorrhage, was also classified as PVL or haemorrhagic PVL in accordance with the work of LEVENE et al. (1983), WIGGLESWORTH (1984) and LAURINI (in press).

RESULTS

Gross postmortem examination of the brain showed no abnormalities in 4 of the 19 infants. The abnormalities found in the remaining 15 infants are summarized in Table 1.

TABLE 1. Abnormalities found on gross postmortem examination of the brain in 15 infants.

		number of abnormalities
Germinal matrix haemorrhage		5
Intraventricular haemorrhage	grade II	4
	grade III	2
	grade IV	3
Periventricular leucomalacia		7
Parenchymal haemorrhage		1
Cerebral edema		2
Subarachnoid haemorrhage		1
Infratentorial haemorrhage		1
Hydrocephalus		1

In seven infants PVL was found; in four of these infants PVL was associated with secondary haemorrhage. Except for one infant with a subarachnoid haemorrhage, all cerebral haemorrhages were diagnosed on sonography by all examiners, this included the infants with PVL with secondary haemorrhage. Non-haemorrhagic PVL, however, was not diagnosed by any of the 5 examiners in two of the three cases in which it occurred (67%). Moreover, in twelve infants, where postmortem examination revealed no PVL, PVL was diagnosed on sonography in one (8%) to six cases (50%) depending on the examiner (Table 2).

TABLE 2. The incidence of false sonographic diagnosis of periventricular leucomalacia (PVL) by five different examiners in twelve newborn infants in whom postmortem examination of the brain revealed no signs of PVL.

Examiner	False positive diagnosed PVL
a	2 (16 %)
b	1 (8 %)
c	5 (41 %)
d	5 (41 %)
e	6 (50 %)

A false diagnosis of PVL on sonography was made in a total of nine of the twelve infants. The postmortem findings in these 9 infants and the false positive diagnosis of PVL made by all five examiners are presented in Table 3.

DISCUSSION

This study clearly demonstrates that the detection of non-haemorrhagic PVL at an early stage by means of sonography with a 5 MHz transducer is very difficult. In 2 of the 3 (67%) cases where non-haemorrhagic PVL was found on postmortem examination, it remained undiagnosed on sonography. In addition to these false negative diagnoses, we found that a disturbing number of false positive diagnoses was made. Some examiners seem to diagnose PVL on sonography too easily, making this diagnosis incorrectly in up to 50% of infants without PVL (Table 2).

Our findings seem to contrast with those of NWAESEI et al. (1984)

TABLE 3. Falsely diagnosed periventricular leucomalacia (PVL) on sonography by five different examiners (a through e) and the postmortem findings of the brain of the infants.

Infant	Examiners					Postmortem findings
	a	b	c	d	e	
I			+	+	+	necrosis
II			+	+		normal
III		+			+	subarachnoid haemorrhage
IV					+	congestion
V	+				+	small (4 mm) GMH
VI					+	normal
VII	+		+	+		congestion
VIII			+	+	+	normal
IX			+	+		grade III IVH

+ PVL diagnosed; GMH germinal matrix haemorrhage; IVH intraventricular haemorrhage

who found a good correlation between sonographic and postmortem findings in infants with non-haemorrhagic PVL. Compared to the study of NWAESEI et al. (1984), however, the infants with PVL in our study died at a much younger age (mean age at death 5 days vs. 66). It is obvious from this difference that NWAESEI et al. (1984) were actually dealing with the more established lesions of PVL including cyst formation. Like us, however, they showed that the diagnosis of non-haemorrhagic PVL was missed on sonography in infants under fourteen days of age. Therefore, the apparently good correlation between sonographic and postmortem findings reported by NWAESEI et al. (1984) is limited to the more developed lesions of PVL seen in older infants and does not hold for newborn infants within the first two weeks of life.

What factors may contribute to the difficulty of diagnosing non-haemorrhagic PVL on sonography in infants of less than two weeks of age? One of the pitfalls appears to be congestion of the brain, seen on postmortem examination as cerebral edema associated with vascular congestion, which was present in two infants in whom PVL was incorrectly diagnosed on sonography (Table 3). This pitfall has been previously discussed by LEVENE et al. (1983), who speculated that echo-reflections from congestion might be misinterpreted as PVL. Another

explanation for the false diagnoses of PVL especially in very immature infants might be that differences in the composition of the fetal brain during the course of pregnancy (WIDDOWSON and DICKERSON 1960, CONDE et al. 1974) result in differences in the sonographic image of the brain. Indeed five of the nine infants in this study where non-haemorrhagic PVL was falsely diagnosed had a gestational age of less than 28 weeks.

When first reported, PVL, on postmortem examination, was described as small white spots in the periventricular region (BANKER and LARROCHE 1962). Nowadays, however, more extensive lesions are seen (HILL et al. 1982, WIGGLESWORTH 1984). Infarction of the periventricular white matter may destroy the brain to such an extent, that the pathologist misinterpretes this as an artefact (WIGGLESWORTH 1984). Such an extensive white matter necrosis was found on gross examination in patient I (Table 3), who showed a rapid clinical deterioration. One examiner considered the sonogram of this infant to be abnormal, though he was unable to indicate the nature of the abnormality. Three other examiners diagnosed PVL in this infant, but their diagnosis was based on echodensity, seen in an areaoutside of the extensive white matter necrosis. They considered the rest of the brain including the area with extensive necrosis to be normal. These diagnoses of PVL were therefore scored as false positives.

Recently, DUBOWITZ et al. (1985) showed that sonographic imaging was well correlated with the various stages of PVL. They used a 5 and a 7 MHz transducer. Our study indicates that if only a 5 MHz transducer is used, PVL without haemorrhage, in its early stage, cannot be diagnosed with high reliability and therefore, should only be diagnosed with great caution. Follow-up scans, showing secondary porencephalic cysts, or postmortem examination of the brain are necessary to confirm an early suspicion of PVL.

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CHAPTER 6: MORPHOLOGICAL FINDINGS IN PLACENTAS OF INSULIN DEPENDENT DIABETIC PATIENTS TREATED WITH CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (CSII)

SUMMARY

Twenty-one placentas from type 1 (insulin-dependent) pregnant diabetic patients, treated with continuous subcutaneous insulin infusion (CSII) were studied morphologically. Despite a near-optimal blood glucose control the placental changes were identical to those previously reported in diabetic pregnancy. The most frequently observed lesion was that of relative placental immaturity; when extensive it was related to antenatal fetal asphyxia. These data indicate that near normoglycaemia, achieved with CSII, does not modify the morphological expression of the disease in the placenta. Furthermore, it highlights the importance of placental development in the context of diabetic pregnancy.

INTRODUCTION

Although the assessment of placental changes in women with diabetes mellitus remains a matter for discussion, there is a widespread notion that these changes are related to a poor metabolic control during pregnancy.

A tight glycemic control during pregnancy can be achieved by means of a continuous subcutaneous insulin infusion (CSII). As a result placentas from these patients should either show no abnormality or only minimal changes.

On this assumption, we examined placentas from CSII controlled diabetic pregnancies.

PATIENTS

Nineteen C-peptide negative, insulin-dependent diabetic women (White class B-D, mean duration of diabetes 11.4 yr), were treated with CSII

during 21 pregnancies. In 8 patients pump-therapy was started before conception, in 12 patients before the 16th week of gestation and in 1 patient in week 23.
Obstetrical history of the patients in whom CSII treatment was started before conception was, on average, poorer than that of the women in whom this treatment was started after conception (i.e. previous unexplained stillbirths or congenital malformations). There were 5 smokers; four women smoked, on average, 10 cigarettes a day and one over 20 a day. The latter women was delivered of an extremely growth retarded fetus. There was no history of alcohol or drug abuse amongst the mothers studied.

MATERIAL AND METHODS

CSII was performed according to the method described by Pickup et al. (1979). In all patients a Mill Hill Infuser, model 1001 AM (Muirhead Medical Products, Ltd, London, UK) was used. To assess the degree of glucose control, 24-hour blood glucose profiles (8 finger-prick samples; hexokinase method) were obtained from all patients every two weeks. Home blood glucose monitoring was performed with Haemo-glukotest 20-800 reagens strips Boehringer-Mannheim, FRC, 4-8 determinations daily. Glycosylated haemoglobin (HbA_{1C}) was measured every two weeks by the colorimetric method of Flückiger and Winterhalter (1976). In all patients the best possible control without hypoglycaemia was aimed for, i.e. blood glucose values between 2.5 and 7 mol/l.

Good metabolic control could be achieved in all patients with a mean blood glucose ($\pm$ 1DS) of 5.3 $\pm$ 1 mmol/l (Table I). On 17 occasions HbA_{1C} values were measured around 10 weeks of gestation that, on average, were somewhat above the normal range (normal range 6-8.5%) (Table II). Mean glycosylated hemoglobin at delivery was 6.4 $\pm$ 1%. In only one patient HbA_{1C} at term was above the normal range. None of the patients suffered ketoacidosis during the study; severe hypoglycemia (defined by the need for assistance by somebody else) occurred 5 times in 4 patients. Two women developed a mild pregnancy induced hypertension (PIH). Polyhydramnios was not seen. There were one spontaneous and ten

induced preterm deliveries (33-37 weeks). On 10 occasions pregnancy was terminated by Caesarean section (CS). The reasons were antepartum late heart rate decelerations (n=4); failure to progress during labour (n=4); repeat CS (n=1) and CS because of recurrent severe haemorrhagic oesophagitis (n=1). The remaining pregnancies lasted till term (≥ 37 weeks).

There were four cases of antepartum fetal asphyxia (late heart rate decelerations) and one patient suffered an unexplained intrauterine death in the 37th week of gestation (birthweight 95-97.7 percentile). According to the Dutch intrauterine growth curves (Kloosterman 1970) 8 of the 21 infants had a birthweigh > 90 percentile (3 > 97.7 percentile).

Table I summarizes the obstetrical data including blood glucose control and fetal outcome.

Placentas from all 21 pregnancies were available for examination. All were processed for light microscopy (LM) and 11 also for electron microscopy (EM). Samples from central portions (maternal side) were taken immediately after delivery and fixed in 2% glutaraldehyde in 0.1 M phosphate buffer, dehydrated and embedded in Epon 812. Sections were cut with the Sorvall MT 5000 microtome and stained with toluidine blue-basic fuchsine (Flores and Hoffman 1981) for LM control and with 1% uranylacetate and lead citrate for EM examination with a Philips EM 201/300 microscope. Following sampling for EM, the placentas were fixed whole in 4% formalin. After fixation, the umbilical cord, membranes and excess blood were removed before the placenta was weighed and sliced into 1 cm thick coronal sections.

Gross anatomical findings were expressed as percentage on affected placental volume. Blocks were taken from umbilical cord (2x), membranes (roll), umbilical insertion and full thickness of macroscopically normal placenta (2x). In addition, a variable number of blocks from abnormal areas were taken. These blocks were embedded in paraffin while extra blocks (2x) from macroscopically normal placenta (maternal side) were embedded in plastic for LM. Paraffin embedded tissue was stained with haematoxylin-eosin. Plastic sections were stained with a modified toluidine-blue-basic-fuchsine stain (Flores and Hoffman, 1981) consisting in shorter staining time for toluidine blue (5 seconds) and basic fuchsine (15 seconds). A group of 21 placentas

TABLE I: CLINICAL DATA REGARDING THE 21 PREGNANCIES (placenta and fetal birthweight percentiles according to the Dutch growth curves (Kloosterman 1970)).

Patient initials	Histol. Class.	White Class.	Placenta weight percentile	Birth-weight percentile	Blood glucose control				Pregnancy induced hyper-tension	Antenatal asphyxia or fetal death(*)	Spontaneous premature delivery	Delivery (week of gestation)
					mean blood glucose during pregnancy (mmol/l)	mean blood glucose during 3rd trimester (mmol/l)	mean HbA_1 during pregnancy (%)	HbA_1 at delivery				
T.V.	1	D	25-50	10-25	5.6	3.8	9.1	7.2	-	-	-	38
A.N.	1	C	50	75-90	7.7	5.7	-	-	+	-	-	36
E.W.	2	C	-	2.2-5	4.7	6.0	7.5	5.8	-	-	-	39
F.S.	1	C	-	50-75	5.2	5.2	8.4	7.3	-	-	-	38
A.M.	1	B	-	10-25	5.7	5.4	8.1	7.6	-	+	-	35
M.d.J.	3	C	50-75	95.97.7	6.0	5.4	9.7	9.3	-	+(*)	-	36
M.d.J.	3	C	75-90	75-90	4.9	5.5	8.2	7.0	-	+	-	33
J.B.	5	C	75-90	50-75	4.4	5.4	7.4	7.6	-	-	-	37
T.J.	5	C	90	95-97.7	5.4	6.3	7.8	7.5	oedema	-	-	34
G.S.	3	C	90-95	75-90	4.8	4.8	7.0	6.9	-	-	-	35
J.R.	6	B	97.7	75-90	4.9	5.0	8.2	8.1	-	-	-	37
F.N.	3	D	10-25	2.3-5	4.8	5.4	6.0	5.6	-	+	-	33
W.B.	1	D	95-97.7	90-95	6.7	6.0	7.2	7.0	-	-	-	37
L.V.	4	C	75-90	95-97.7	5.4	4.0	6.7	5.6	-	-	+	33
T.V.	1	R	75-90	50-75	4.6	5.0	6.6	5.9	-	-	-	37
A.J.	5	B	90-95	90-95	6.5	6.8	6.6	6.9	-	-	-	37
E.P.	1	B	90-95	$\geq$ 97.7	6.1	6.1	6.7	6.6	-	-	-	35
K.V.	4	B	90-95	$\geq$ 97.7	6.2	6.2	6.4	5.8	-	-	-	38
G.W.	2	C	75-90	75-90	5.2	5.8	6.9	5.8	-	-	-	38
S.d.V.	3	C	25	25-50	4.8	6.0	6.2	6.0	-	+	-	35
H.v.d.B.	3	D	90-95	$\geq$ 97.7	6.4	6.3	7.3	6.3	+	-	-	36

from uneventful pregnancies with the same gestational range were used as control. These placentas were similarly trimmed and weighed before and after formalin fixation. Otherwise the same LM protocol was followed. This protocol is routinely used for all placentas studied at our department except for EM and plastic LM which are used more selectively. Histological assessment was carried out with special emphasis on the following aspects: degree of placental relative immaturity and villous oedema - fetal stem arteries abnormalities - fetal villous capillaries position, degree of dilatation and endothelial abnormalities - presence of syncytial necrosis, syncytial knots - abnormal cytotrophoblast - increased villous stromal cellularity with/without stromal fibrosis - trophoblastic basement membrane thickening - "blebs" (Diara Haust, 1981) and microinfarction. Placental relative immaturity for gestation was defined by the following parameters: large villi - continuous syncytial trophoblast - increased cytotrophoblast (Langhans cells) - non-dilated villous capillaries - increased stromal cellularity.

The placentas were divided into 3 groupes according to the degree of relative immaturity present; (+) occasional immature villi, (++)

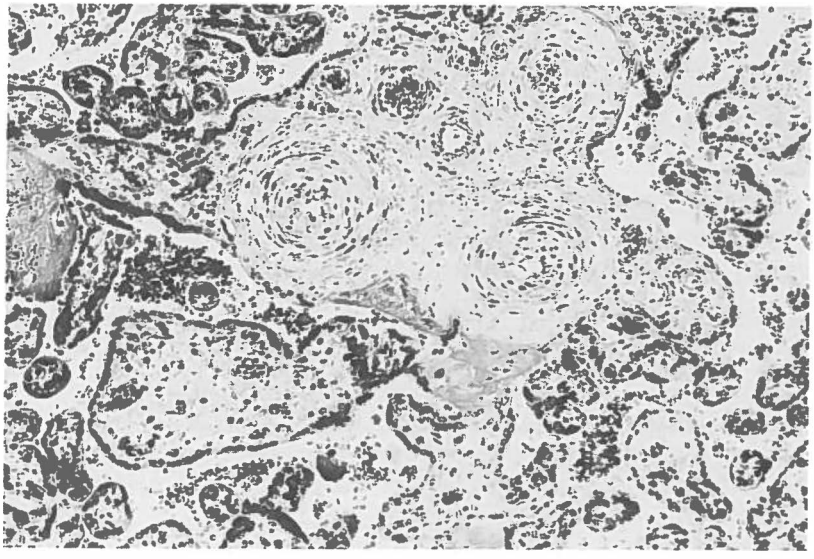

Fig. 1. High magnification of small chorionic arteries with variable degree of "endarteritis" (Hematoxylin-eosin, x 140).

several foci of villous immaturity, (+++) multiple foci present in all sections. For each such group the presence or absence of widespread fetal vessel "endarteritis" (Fig. 1) was recorded. This resulted in a histological classification (HC) consisting of 6 groups where the different degrees of villous immaturity was related to the presence (HC 4/5/6) or absence (HC 1/2/3) of vascular changes (Table II).
Table II summarizes the relationship between the HC and obstetrical data, blood glucose control and fetal outcome.
This morphological classification was made by the pathologist without his having any knowledge about the course of pregnancy and fetal outcome. In three cases we reviewed formalin-fixed, paraffin embedded placentas from pregnancies previous to CSII control.

RESULTS

Gross examination

The CSII placentas (n=18) weighed between 270 and 740 gr with a mean of 552 gr. The CSII placentas (n=14) from pregnancies of 36-41 weeks weighed between 450 and 740 gr with a mean of 593 gr. There was no significant difference in placental weight between patients in whom CSII was started before or after conception.
Three placentas showed macro-infarctions (<5% placenta volume).
No lesions were seen in the rest. One umbilical cord showed a single umbilical artery unassociated to congenital anomalies in the infant.

The control placentas weighed between 260 and 560 gr (mean 422 gr) before fixation and between 260 and 540 gr (mean 442 gr) after fixation. There was no statistical difference in the placenta weight before and after fixation in formalin (Student t-test $p>0.2$). There was a statistical difference between the weight of CSII and control placentas (Student t-test $p<0.01$).
The control placentas failed to show abnormal features on gross examination.

TABLE II: RELATIONSHIP BETWEEN PLACENTA HISTOLOGY AND CLINICAL FINDINGS

histology classification	placental morphology			birthweight percentiles		blood glucose control				pregnancy induced hypertension	antenatal asphyxia or fetal death(*)	White Class		
	villous immaturity	vessel lesions	no. of placentas	<10th	≥90th	mean blood glucose (mmol/l)	mean glucose amplitude (mmol/l)	mean HbA_1 (%) 1st trim.	mean HbA_1 (%) at delivery			B	C	D
1	+	-	7	-	2	5.9	5.2	9.4	6.6	1	1	1	3	3
4	+	+	2	-	2	5.8	5.4			-	-	1	1	-
2	++	-	2	1	-	4.9	5.6	7.6	6.7	-	-	-	2	-
5	++	+	3	-	2	5.4	5.3			-	-	1	2	-
3	+++	-	6	1	2	5.3	5.6	8.7	7	1	4(*1)	-	4	2
6	+++	+	1	-	-	4.9	4.7			-	-	1	-	-

Light microscopy

The CSII placental changes showed a marked degree of variability. Not all abnormalities were present in every placenta, still there were no placentas within normal limits, when compared to the control placentas.

Vascular changes

Variable degrees of "endarteritis" of small chorionic arteries were seen in nine placentas. In addition, severe changes were present in 6 cases (Table II) (Fig. 1). The latter included three placentas with fetal stem artery obliterative endarteritis and a case with fetal artery thrombosis. Placentas with "endarteritis" also had fetal capillaries with prominent endothelium and partial, or occasionally, total occlusion of the lumen. Villous vascularity showed considerable variability. Mainly in relation to villous immaturity there was a predominance of non-dilated fetal capillaries that tended to be centrally located (Fig. 2). In such areas the vasculo-syncytial membranes were poorly developed. On paraffin embedded material a number of placentas showed what impressed as hypovascular villi. Still LM on plastic sections demonstrated the presence of multiple small fetal capillaries. These were frequently located in the periphery of the villi penetrating the trophoblast as can be seen in early sprout formation (Fig. 2).

Villous immaturity

All CSII placentas showed some degree of relative villous immaturity with about an equal number of cases with limited (+) (Fig. 3) as extensive (+++) (Fig. 4) immaturity (Table II). Control placentas failed to show villous immaturity as here defined. Manifest villous oedema was seen in only two cases although occasional oedematous villi were present in ten placentas. Most probably related to villous immaturity and oedema, there were numerous Hofbauer cells as well as manifest prominence of Langhans cells with occasional mitosis. Increased syncytial knots, focal syncytial necrosis, stromal fibrosis and excess of villous fibrinoid necrosis were also frequent but showed notable variation from

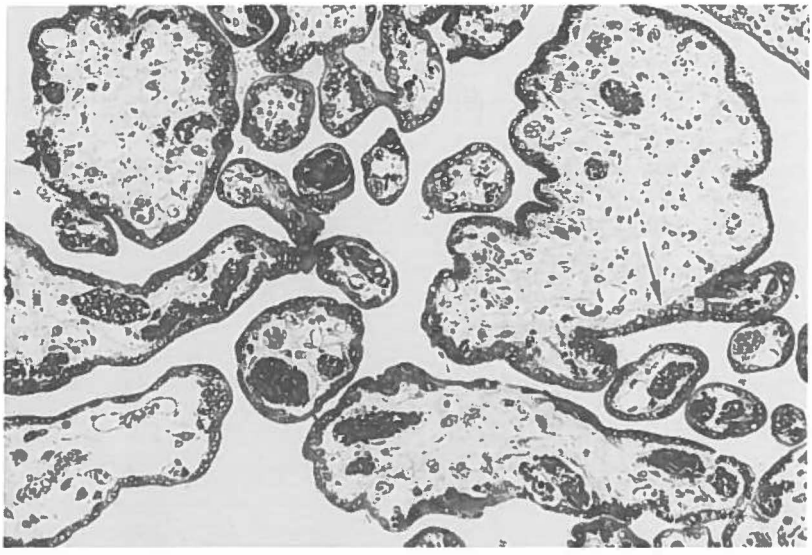

Fig. 2. High magnification of placental villi. Note large villous with prominent cytotrophoblast (thick arrow), centrally placed fetal vessels and multiple non-dilated fetal capillaries under the trophoblast (thin arrow). (Plastic embedded, hematoxylin-eosin, x 140).

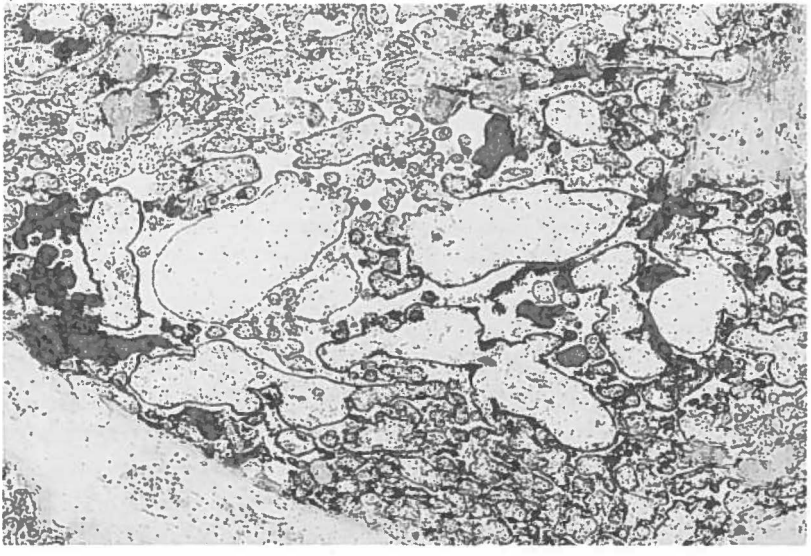

Fig. 3. Focal area of villous immaturity as observed in grade 1 placental relative immaturity (hematoxylin-eosin, x 35).

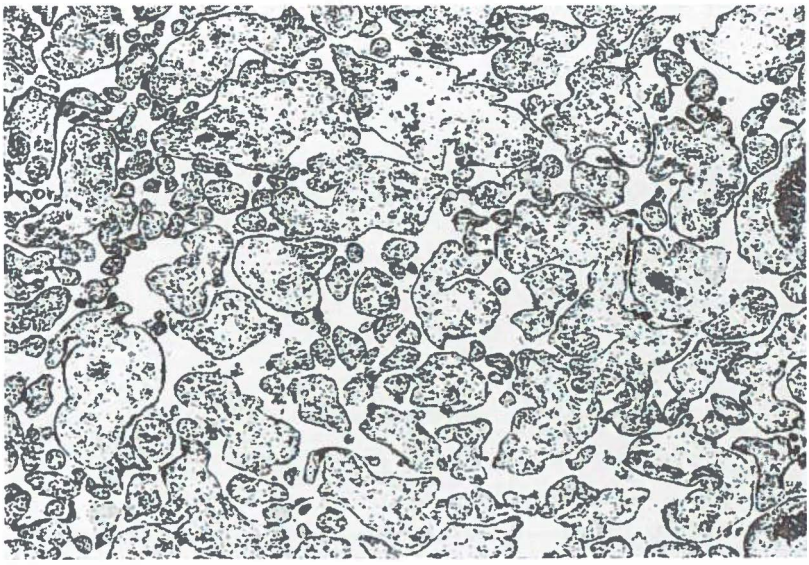

Fig. 4. General view of the diabetic placenta at term (≥ 37 weeks). Predominance of large villi intermingled with the normal small type (hematoxylin-eosin, x 56).

case to case.
The two cases with CSII control during two consecutive pregnancies showed the same degree of relative immaturity in each of the placentas. One case had extensive (+++) changes while the other showed only a limited (+) degree of villous immaturity in both placentas.
Review of placentas from pregnancies previous to CSII treatment showed similar changes as compared to the placentas of CSII treated pregnancies.

Electron microscopy

This confirmed the LM findings. In addition, the syncytiotrophoblast showed focal syncytial necrosis and frequent dilation of rough endoplasmic reticulum together with an increased number of secretory granules. All cases showed focal thickening of the trophoblastic basement membrane including foci with a lamellated structure (Fig. 5). Langhans cells were always prominent, frequently with active cytoplasma and, occasionally, with mitosis (Fig. 5 and 6). Villous vessels consisted, predominantly, of small peripheral fetal capillaries as was

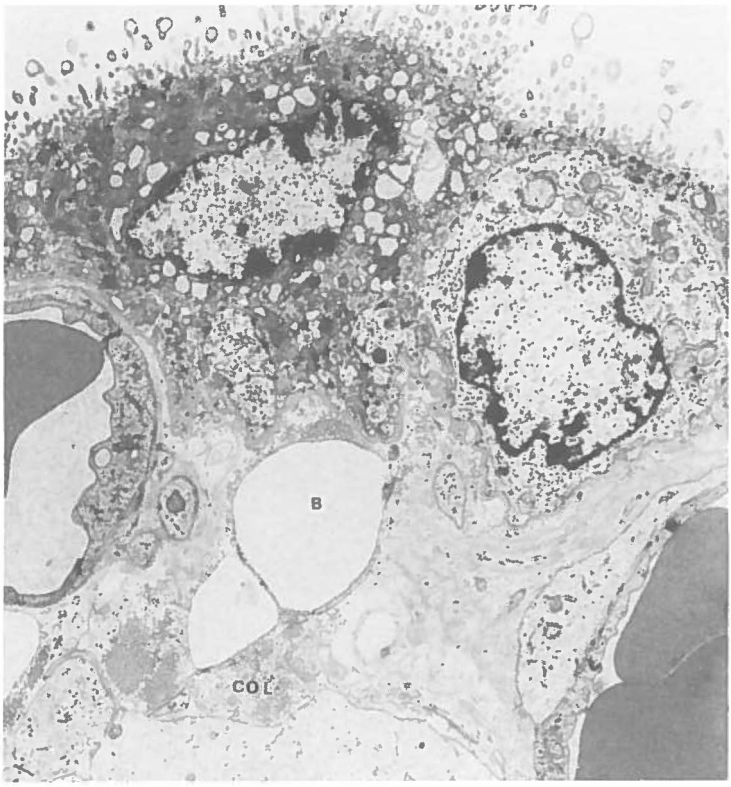

Fig. 5. Electronmicrograph of a terminal villous showing presence of "blebs" (B) in the villous stroma as well as increased collagen (Col). The basal membrane is wavy and focally lamellated (x 8628).

seen with LM (Fig. 6). These capillaries showed occasional hyperplasia of endothelial cells, with frequent abundant cytoplasma, variable narrowing of the lumen and distinct pericytes (Fig. 7).
Prominent endothelial cells with narrowing of the lumen and hyperplasia of muscular layer was also seen in small branches of chorionic arteries. Well-formed vasculo-syncytial membranes were occasionally present. The villous stroma showed variable increase of cellularity mainly due to stromal cells. Many of these presented with the characteristics of active fibroblasts and were usually surrounded by collagen. Stromal "blebs" were seen in 7 of the 11 placentas studied with EM (Fig. 5). In one case similar changes were present in the syncytiotrophoblast.

Fig. 6. Tissue as in fig. 5. Note prominent cytotrophoblast (C) and non-dilated fetal capillary (F) "invaginating" into trophoblast (x 5660).

Glycogen was not a common feature of these placentas and was mainly seen in stromal cells.

On LM and EM there were no significant differences between the type/degree of placental abnormalities seen with pre- or post-conceptional CSII control.

The relationship between placental histology and clinical findings is shown in Table II. The degree of villous immaturity was not related to the White class, nor to blood glucose control during pregnancy. Despite some elevation of the HbA_{1C} mean values in the first trimester there was no relation between diabetic control in early pregnancy and the histological classification (Table II). In 3 out of 5 cases with severe villous immaturity (HC 3) early pregnancy HbA_{1C} values were in the range of normal. There was no statistically significant difference in

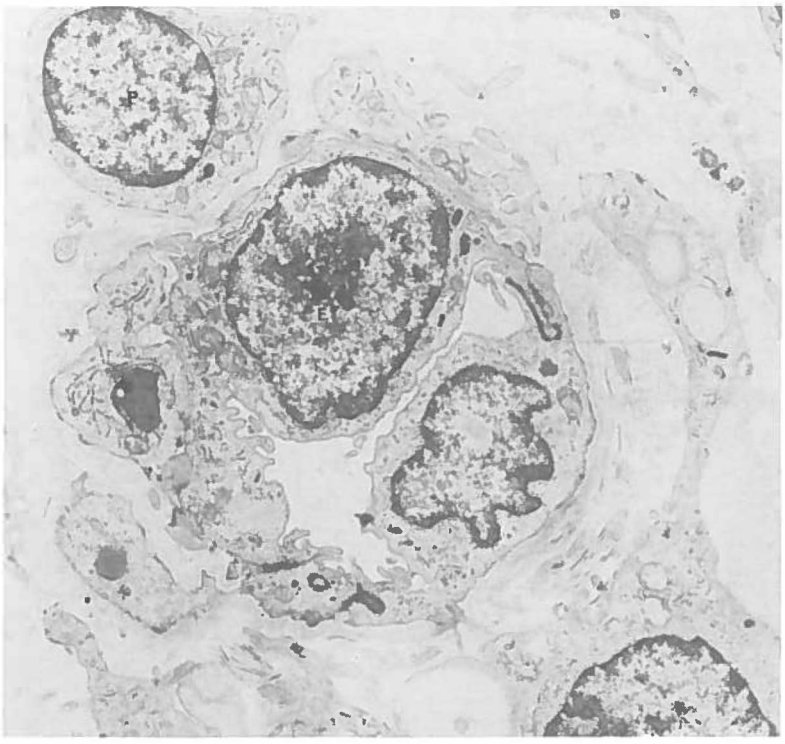

Fig. 7. Tissue as in fig. 5. Villous capillary with prominent endothelial cells (E) and slitlike lumina. Note prominent capillary-associated pericyte (P) (x 12992).

metabolic control between the 3 groups of villous immaturity (Wilcoxon test for unpared data). Antepartum asphyxia (late heart rate deceleration; n=4) and "unexplained fetal death" (n=1) were seen in 4 out of 7 cases with severe villous immaturity, in contrast to 1 out of 14 less prominent immature findings (Table II).

There was one patient with an unexplained fetal death at 36 weeks, in a previous pregnancy. This time she was delivered at 35 weeks, without signs of fetal asphyxia; the placenta, however, showed a severe villous immaturity.

The outcome of pre- and postconceptional CSII treatment as measured by birthweights centiles, occurrence of antepartum asphyxia and duration of pregnancy was almost identical in both groups.

vessel lesions were only seen in White class B and C patients; none of the 5 placentas of women with type D diabetes showed these lesions. The incidence of PIH and fetal growth retardation was too low to warrant conclusions. Large-for-dates infants were seen in all categories of placental abnormalities.

DISCUSSION

The assessment of morphological changes in placentas from women with diabetes mellitus has been the cause of much controversy (Daria Haust 1981; Teasdale 1981). The association with hypertensive disease of pregnancy and/or intrauterine death as well as the limited numbers included in different series have added to the confusion. Still a somewhat characteristic morphological pattern has been reported in maternal diabetes mellitus (Fox 1969). The placental changes both with LM and EM found in this study are identical to those previously reported (Daria Haust 1981). The incidence and degree of both hypertensive disease of pregnancy and intrauterine death was too low to have played a significant role with regard these morphological changes. Although there was considerable variability in the presence and extent of the abnormalities, no placenta showed such limited changes that it could be considered within the range of normal. The marked degree of variability might be an important reason for the inconsistency in the literature as with regard to placental changes associated to diabetes mellitus (Daria Haust 1981; Teasdale 1981). A less comprehensive placenta protocol than the one used for this study, could also have had a significant influence on the results in view of the referred morphological variability.

Furthermore there was not only a statistical significant difference in weight between control and CSII placentas, but this placentomegaly was accompanied by a fetal macrosomia.

Although the pathogenesis of these morphological changes remains obscure, so far the general consensus has been that they are associated with a poor metabolic control during pregnancy. This despite the findings by Jones and Fox (1976) on LM and EM studies, carried out in

7 placentas from well controlled gestational diabetic patients that showed identical changes as found in overt diabetes. Their findings, as well as those of the present study, demonstrate that adequate blood glucose control does not prevent the development of placental abnormalities. This is also indicated by the fact that placentas from pregnancies controlled by CSII failed to show a significant morphological difference as to those from previous pregnancies.

Furthermore, not only placental but also fetal and neonatal abnormalities occur in the context of tight glycemic control achieved with CSII as we have previously reported (Laurini, Visser and Ballegooie 1984). These findings were identical to those previously reported by others (Daria Haust 1981). Other recent reports have also drawn attention to the fact that well controlled diabetic pregnancy (CSII) does not prevent macrosomia (Knight, Worth and Ward 1983) nor hypertrophic cardiomyopathy (Verhaaren et al. 1984).

The most frequently observed lesion was that of relative placental immaturity; when extensive it was related to antenatal fetal asphyxia. This suggests that severe villous immaturity has a detrimental effect on third trimester fetomaternal oxygen exchange. This is probably the result of diffusion limitations due to the increased distance between intervillous space and fetal capillaries because of continuous syncytium trophoblast, increased cytotrophoblast and villous oedema as well as the non-dilatation of the fetal capillaries. Birthweight percentiles were not related to the degree of placental immaturity. This can, at least in part, be explained by the fact that glucose and other nutrients are (partly) actively transported over the placenta. The discrepancy between glucose and oxygen transport over these immature placentas may cause fetal lactate accumulation and therefore play a role in the socalled "unexplained" death in utero. In the two cases reported in this study, the most obvious placental abnormality was indeed a severe placental immaturity.

In conclusion: This study demonstrated that a tight metabolic control is no guarantee that complications of diabetic pregnancy will be avoided. In contrast, our findings strongly suggest that a near-optimal glycaemic control achieved with CSII does not affect the morphological expression of the disease.
Such control may influence clinical outcome but not the disease process

itself. Our findings also suggest that placental development is an important factor in the context of diabetic pregnancy.

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CHAPTER 7: EXPERIMENTAL DIABETES IN PREGNANCY: FETAL AND PLACENTAL PATHOLOGY IN WISTAR RATS.

INTRODUCTION

In chapter 6 we have observed that both obstetrical and morphological abnormalities, characteristic for insulin-dependent diabetes mellitus, persist even when the blood glucose is tightly controlled by continuous subcutaneous insulin infusion (CSII).
Therefore the question arises, which factor(s), other than the blood glucose concentration, may be involved in the genesis of these abnormalities. For the study of that question an animal model, amongst others, is needed. In the present chapter we investigate whether the rat is a suitable model for the evaluation of feto-placental pathology in different conditions of maternal glycaemic control.

MATERIAL AND METHODS

Rats (n=22) of the Wistar derived strain at the Groningen University were made pregnant at the age of 4 months. The day after copulation was called day 0 of pregnancy. On day 5 of pregnancy streptozotocin (75 mg/kg; in 0.1 M sodium-citrate, UpJohn Co.) was injected i.v. in 18 rats; control rats (n=4) were injected with sodium-citrate only. On day 6 a blood sample was taken from the orbital plexus and an Alzet osmotic minipump (model 2001; Alza Co.), containing a saline solution of 100 U/ml of semisynthetic human insulin (Actrapid, Novo Industries), was subcutaneously implanted in 11 of the streptozotocin-treated rats. The infusion rate of the minipump was 1 µl per h (the rats therefore were infused with 2.4 U per day) for a period of 7-8 days. On day 14 a second blood sample was taken and the minipump was replaced by a new one.
On day 21 (1 day before the expected day of parturition) the rats were decapitated and blood was sampled from the trunk. The number of implantation sites in the uterus was counted and of each feto-placental unit the position was noted and numbered (starting at the ovarian end of the right hern). Fetuses were assessed for viability. Placenta and fetuses were weighed and the (crown-rump) lenght of each fetus measured. After weighing samples from the first two odd-numbered placentas from each litter were processed for electronmicroscopic examination (see Chapter 6). The

metrial glands were dissected and together with the placentas fixed in buffered formalin. A transversal section from the metrial gland and a whole coronal section from the placenta at the site of insertion of the umbilical cord were embedded in paraffin and stained with haematoxylin-eosin, Masson's tri-chrome, Perl's (iron) and Von Kossa (calcium). All resorptions (n=14) were fixed in buffered formalin and a whole central coronal section was embedded in paraffin and stained with haematoxylin-eosin and Masson's trichrome. The odd-numbered fetuses (n=38) were chilled in ice and then fixed in 96% ethanol for staining with Alizarin Red S. After fixation of the fetuses in 96% ethanol for 3 weeks or more they were submerged in acetone for 12h and subsequently in a 1% (w/v) solution of KOH, which also contained 5 mg/l Alizarin Red S, for 2 consecutive periods of 24 h. Then the fetuses were rinsed in aqua dest. for 2.5 h. Clearing of the tissue was performed in benzylalcohol/ glycerol/70% ethanol (1:2:2) for 24 h. The stained and cleared fetuses were stored in glycerol (with a crystal of thymol). Once stained thirty-four fetuses were radiographed with a Faxitron series-43805N, Hewlet-Packard).

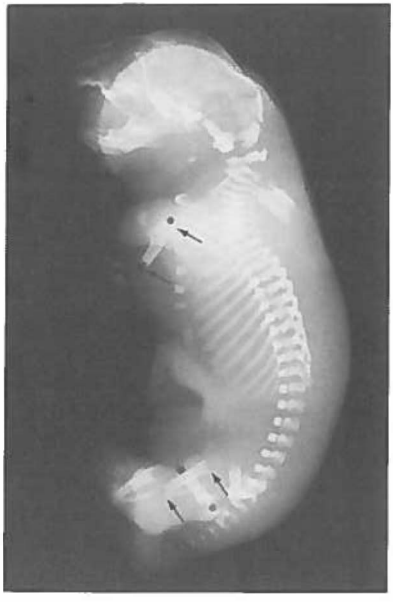

Fig. 1. Radiograph of a control fetus. Arrows indicate to calcified portion of the humerus and femur used for assessment of skeletal development.

The even numbered fetuses were fixed in buffered formalin. All fetuses were inspected for congenital malformations on gross examination, after Alizarin Red S staining and by radiography with emphasis on micrognathia and caudal dysgenesis (ERIKSSON et al. 1982). Skeletal development was assessed by measuring the calcified portion of the humerus and femur (Fig. 1). Fetuses (n=20) from control animals were used to establish the normal range for these bones at 21 day gestation.

RESULTS

All 7 diabetic pregnant rats, not treated with insulin, died before the planned day of sacrifice, of the insulin treated diabetic rats 6 out of 11 died, of the 4 control rats none (Table 1).

TABLE 1. Number of mothers, implantations, control and treated animals.

	NUMBER OF MOTHERS	MATERNAL DEATHS	IMPLANTATIONS	RESORPTIONS	LIVE FETUSES
Control	4	0	43	0	43
Streptozotocin	7	7	-	-	-
Streptozotocin-pump	11	6	41	14	27

The mean number of live fetuses per mother on day 21 was higher in control mothers (10.8 $\pm$ 0.6) than in the diabetic mothers (5.4 $\pm$ 1.6) (Mann-Whitney U-test: $p<0.05$), due to the large number of resorptions (intrauterine deaths) and not to a reduction in the number of implantations.
In Table 2 the glucose plasma concentrations (g/l) on day 6 (after streptozotocin treatment, but before implantation of the minipump) and on day 14 and day 21 are reproduced. Glucose levels in the streptozotocin treated rats were on day 6 higher than in control rats, but were not significantly different on day 14 and 21. At the latter ages there were, however, large variations in glucose concentrations in the diabetic rats.
It can be inferred that a constant rate of subcutaneous infusion of insulin in diabetic pregnant rats reduces the glucose levels to near the normal range, but the great variability does not suggest a "tight metabolic control".

TABLE 2. Maternal serum glucose concentrations in control and insulin-treated diabetic rats (g/l).

	CONTROL (n=4)	DIABETIC (n=5)
glucose day 6[a]	1.00 ± 0.11	8.41 ± 1.65
glucose day 14	1.14 ± 0.06	2.11 ± 1.21
glucose day 21	0.90 ± 0.05	1.66 ± 0.70

a Mann-Whitney U-test: $p<0.05$

Table 3 summarizes the results of the developmental assessment of the offsprings from control and diabetic rats.

TABLE 3. Developmental assessment of live fetuses from control and insulin-treated diabetic rats.

	CONTROL	DIABETIC
Placental weight (g)	0.49 ± 0.01	0.46 ± 0.02
Fetal weight (g)[1]	4.74 ± 0.06	3.68 ± 0.13
Fetal length (cm)[1]	3.95 ± 0.03	3.56 ± 0.06
Humerus length (mm)[2]	3.748 ± 0.153	3.035 ± 0.295
Femur length (mm)[2]	2.722 ± 0.190	2.096 ± 0.330

1 Unpaired, two-tailed Student's t-test: $p<0.001$
2 Unpaired, two-tailed Student's t-test: $p<0.002$

The live fetuses of the control group were heavier and longer than the fetuses of the diabetic group. Furthermore assessment of intrauterine skeletal growth (humerus-femur) confirmed the occurrence of intrauterine growth retardation in the offsprings of insulin-treated diabetic mothers.

No malformations were observed in either the control or the insulin-treated diabetic group.

Lightmicroscopic examination of the placentas from the offsprings of control rats were conform the morphological description reported on the rat placenta by DAVIES and GLASSER (1968). This included the presence of atrophy of the decidua basalis with focal, sometimes extensive, lysis of

"glycogen cells" resulting in cyst formation. These placentas failed to showed significant iron and/or calcium deposits.

In the placentas from the diabetic group there were several lesions not present in the control placentas. The abnormalities consisted of: a) areas of pyknosis of the trophoblast with congestion of maternal spaces, b) foci of alternation and atrophy of trilaminar trophoblast, c) focal deposition of iron and/or calcium, mainly in the labyrinth. The extent of the abnormalities showed considerable variability within the experimental group, but all the placentas in this group showed them while the placentas of the control group did not.

Histologic examinations of the resorptions demonstrated a complete absence of the fetus in all cases together with degenerative changes in the placentas. The only difference was that resorptions showed marked degenerative changes with extensive iron and calcium deposition.

The metrial glands were considered to be the equivalent of the placenta bed in human pregnancies since it is the site where the smaller branches of the uterine artery pass into the decidua. Figure 2 illustrates the normal histological appearance in a control case with wide

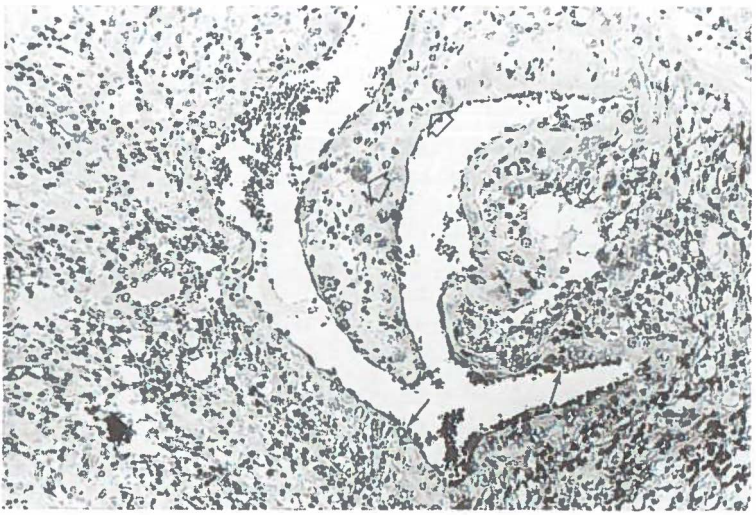

Fig.2. Rat placentation at 21 days in control case; spiral artery converted into wide sinus with trophoblast lining the lumen (small arrow) and embedded in the wall (large arrow). Note normal round cell infiltration and degenerative changes with optically empty glycogen cells (black arrow). Hematoxylin-eosin (x 140).

open vessels allowing for the necessary maternal blood supply to arrive to the maternal spaces in the placenta.
Histological examination of the metrial glands from the diabetic group showed a less prominent or focally absent intravascular migration of trophoblast associated with a marked narrowing of the arterial lumen (Fig. 3).

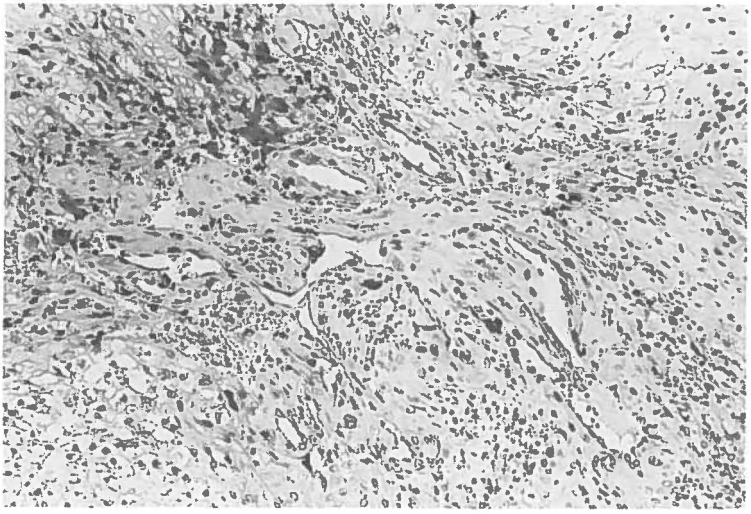

Fig. 3. Rat placentation at 21 days in diabetic case: spiral arteries with narrow lumen, poor trophoblast infiltration and diffuse hyaline changes in wall. Hematoxylin-eosin (x 140).

The metrial glands corresponding to the resorptions showed similar changes only much more severe.

Electronmicroscopic findings on placentas from the control group were in accordance with those reported by ENDERS (1965) and DAVIES and GLASSER (1968). Figure 4 illustrates the normal pattern seen in the labyrinth of the chorioallantoic placenta in control cases. Electronmicroscopic examination of placentas from the diabetic group showed diffuse degenerative changes with a loss of the distinctive trilaminar pattern of the labyrinthine septal with marked increase in the amount of glycogen and of the number and size of lipid droplets (Fig. 5). Furthermore these placentas also showed areas with presence of fibrinoid material, cell debris and occasional polymorphonuclear leukocytes in maternal blood spaces.

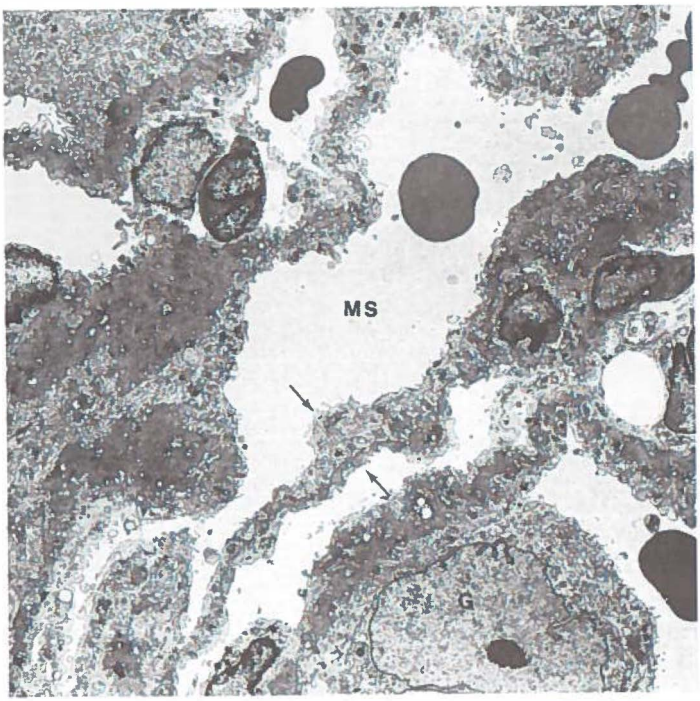

Fig. 4. Electron micrograph of labyrinth of rat placenta at the 21st day in control case. Note the maternal blood space (MS), labyrinthine septa composed by a three layer trophoblastic epithelium (arrows), a giant cell (G) of layer 1 and spread lipid droplets (x2087).

DISCUSSION

Although it was not possible to include a group of untreated diabetic rats the findings in the control and treated group are important for the study of a) rat placentation b) intrauterine growth and its deviations and c) diabetic complications in the absence of tight metabolic control.

In our experimental model the use of an insulin pump did not result in a tight metabolic control during organogenesis. Nevertheless there were no malformations seen in the diabetic group. These data can be compared to that of ERIKSSON et al. (1983). Their rats were made diabetic by a single injection of streptozotocin (SZ) preconceptionally, immediately followed by insulin treatment that was interrupted at different times for a 2-day period in the first half of pregnancy. The

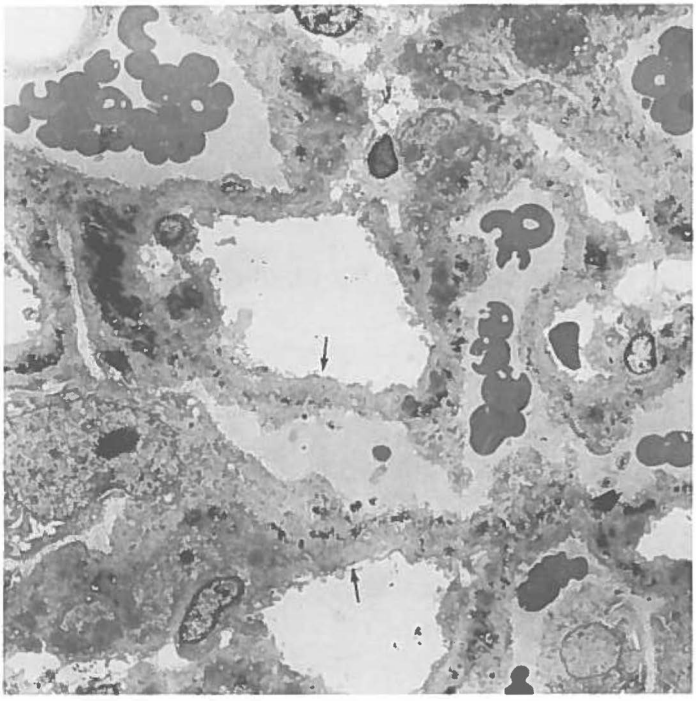

Fig. 5. Electron micrograph of labyrinth of rat placenta at the 21st day in diabetic case. Note the blurring of trilaminar trophoblast (small) arrows) with extensive deposition of glycogen (large arrows) and presence of multiple large lipid droplets (x1386).

interruptions on day 6-7 resulted in only 4 malformed fetuses out of 77. No skeletal malformations were seen in a total of 75 fetuses after interruption of insulin treatment on either days 8-9 or 10-11. However the latter groups showed a high rate of resorptions. In agreement with the results of BAKER et al. (1981) they suggest that the "teratogenic" period in the diabetic rat seems to last up to 8 days after conception. In our model the overt diabetic condition lasted only for 1 day towards the end of the teratogenic period. As with regard the variation in glucose concentration after implantation of the insulin pump one must bear in mind that insulin treatment also failed to completely normalize glycaemia in the model reported by ERIKSSON et al. (1983).

These results suggest that the teratogenic effect of diabetes is not only dependent on the time in gestation but also on a prolonged and substantial increase of the glucose blood level. On the other hand the avoidance

of the complication of intrauterine deaths (resorptions) and intrauterine growth retardation (IUGR) is dependent on a tight metabolic control.

"Pseudo"growth retardation due to diabetes-related delay in implantation seems unlikely. Such a phenomenon could not be demonstrated in a detailed analysis of pregnancy in the spontaneously diabetic Chinese hamster (FUNAKI and MIKAMO 1983). Moreover growth retardation also occurs when in vitro culturing normal postimplantation rat embryos under conditions of high glucose/low insulin concentrations (COCKROFT and COPPOLA 1977, HORTON and SADLER 1983).

Today the impairment of growth in the diabetic rat is considered a multifactorial process (ERIKSSON et al. 1984).
Our findings on vascular trophoblast invasion in the rat metrial gland vasculature (placenta bed equivalent) of the control group are similar to those described by PIJNEBORG et al. (1981) in the hamster. The morphological abnormalities seen by us in the diabetic group can reduce UBF and result in IUGR. These findings also suggest that our experimental model can be of use in the experimental study of the pathogenesis of IUGR, mainly as with regard that which results of an abnormal uteroplacental circulation.

PRAGER et al. (1974) and BROWNSCHEIDLE and DAVIES (1981) have reported cystic lesions in the basal zone of the placentas from diabetic rats. Our findings as well as that of others (DAVIES and GLASSER 1968) indicate that these changes are commonly seen in normal rat placentas after day 18th of the gestation. Nevertheless the placenta from the diabetic group did show significant changes as have been reported in rat (23, 24, 25 days gestation) and human placentas (>42 weeks gestation) from prolonged pregnancies (THLIVERIS 1976, THLIVERIS and BASKETT 1978).

The rat pregnancy corresponds, approximately to the first 60 days of the human pregnancy. In this context early growth delay manifest by the end of week 7 has been reported in the human in type-1 diabetes (PEDERSEN and MÖLSTED-PEDERSEN 1981). Furthermore in Chapter 4 this author has suggested that pathology of the utero-placenta circulation (spiral arteries) with resulting placenta ischaemia can be seen also during the embryonic period in human material. In addition recent work of GERRETSEN et al. (1981) and ROBERTSON et al. (1981) has shown similar changes in the spiral arteries of cases of fetal growth retardation.
In humans there is strong evidence that abnormal uterine blood flow

(UBF) will have a detrimental effect on fetal growth (O'SHAUGHNESSY 1981).

Therefore it is of interest to point out that senescent changes are recognized in both the human placenta and uteroplacenta circulation before term (as discussed by ROBERTSON et al. 1981). It may be that a basic defect established early in pregnancy gives rise to an abnormal UBF secundary to an abnormal uteroplacental circulation that results in an IUGR.

We suggest that when the diabetic condition is severe enough it frequently results in an intrauterine death (resorption). In the presence of a less severe or partially controlled diabetes there is an early growth delay, of possible vascular origin, in both animals and humans. In the human this can be followed by an abnormal development of the placenta (relative immaturity) an macrosomia as result of either a compensatory mechanism or the abnormal development of the placenta. Abnormalities in the development of the haemochorial placentation must be considered when studying the pathogenesis of the complications of diabetic pregnancy.

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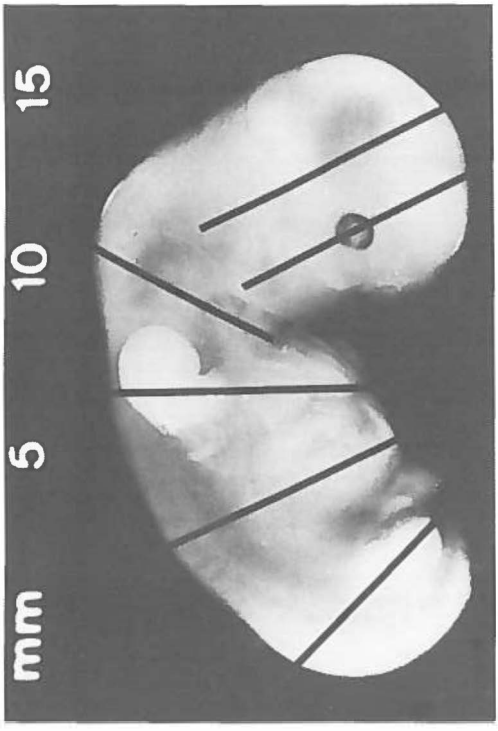
APPENDIX 1: Standard transversal planes of section for specimens up to 4 cm in crown-rump length.



APPENDIX 2: Protocol for the examination of first trimester specimens.

LMP..... G..... P..... Abortions Sex Gestation Diagnosis

Certain ☐
Uncertain ☐

A1 ... .
A2 ... .
A3 ... .
more than 3 ☐

Family history: (malformation, genetic disease, other) NO ☐ YES ☐ Specify:.....
.....

Maternal disease:
Hypertension ☐
Diabetes ☐
Smoking(1), Alcohol(2), Drugs(3) ☐
Other.....
.....

Present pregnancy:
Hemorrhages ☐
Hypertension ☐
Infection ☐
Other.....
.....

Echography:
Gestation.....
Abnormalities.....

AFP:
Maternal serum.....
Amniotic fluid.....

Hormones:
HCG..... ☐
Progesterone..... ☐
Other.....

Amniocentesis:
Cytogenetics.....
Biochemistry.....
Other.....

Chorion villous biopsy:
Week.....
Result.....

Decidua:
Hemorrhage ☐
Others.....

Gestational sac:
Intact.....diameter
Ruptured ☐

Umbilical cord:
NO ☐
YES ☐ .....

Chorion:
Hydropic degeneration: Focal ☐ Diffuse ☐
(vesicles)

Membranes:
NO ☐
YES ☐
Yolk sac.....

Embryo: (1-30 mm CR)
Absent..... ☐
Normal..... ☐
Fragmented..... ☐
Macerated..... ☐
Disorganized growth:
G1 G2 G3 G4
Malformed.....
.....
.....
.....

Fetus: (> 30 mm CR)
Absent..... ☐
Normal..... ☐
Fragmented..... ☐
Macerated..... ☐
Malformed.....
.....

Placenta:
Umbilical cord:.....
Membranes:.....
Chorion:.....

APPENDIX 2

HISTOLOGY

Decidua

Cast ☐ Necrosis/inflammation ☐

Trophoblast invasion (vascular): Yes Haemorrhage ☐

No ..... Other .....

Placenta

Umbilical cord

Nr.vessels: edema ☐ other:.....

Inflammation embryo ☐

Yolk sac:.....

Membranes:

Inflammation: ☐ Other:.....

Placenta

normal development Yes edema ☐ haemorrhage:

No ..... hydropic deg. ☐ (cisterna) intervillous ☐

Nucleated erytrocytes ☐ intravillous ☐

Fibrosis ☐ trophoblast: ischaemia ☐

Mineralization (calcium-iron) ☐ hypoplasia ☐ infarct ☐

hyperplasia ☐ villitis ☐

Pervillous fibrin ☐ stromal trophoblastic inclusions ☐ others .....

stromal trophoblastic cells ☐ .....

fetal vessels ....

....

Embryo-fetus

Developmental assessment: .....

.....

Histology: .....

.....

.....

.....

Diagnosis: .....

.....

.....

APPENDIX 3: Working classification for the morphological assessment of first trimester specimens.

Group 1: Changes secondary to intrauterine death

Group 2: Extensive retroplacental and decidual haemorrhage with or without extension into the intervillous space

Group 3: Simple hydropic abortion

Group 4: Partial or complete mole

Group 5: Extensive ischaemia and infarction

Group 6: a) Extensive polymorphonuclear infiltration around foci of feto-maternal haemorrhage

b) Intervillitis (extensive polymorphonuclear infiltration of the perivillous fibrin)

APPENDIX 4 MORPHOLOGICAL CLASSIFICATION OF GESTATIONAL TROPHOBLASTIC DISEASE

1. PLACENTA SITE REACTION
   (a) NORMAL
   (b) EXAGGERATED - "TROPHOBLASTIC PSEUDOTUMOR"

2. HYDATIDIFORM MOLE
   (a) PARTIAL
   (b) COMPLETE — NON-INVASIVE / INVASIVE

3. PERSISTENT TROPHOBLASTIC DISEASE

4. CHORIOCARCINOMA
   (a) GESTATIONAL CHORIOCARINOMA IN SITU

simple hydropic abortion.* In these cases it is rarely possible to see vesicle formation, naked eye, and microscopically, although there is hydropic change, the trophoblast surrounding the hydropic villi is usually atrophic. No trophoblastic hyperplasia is found. A variety of chromosomal abnormalities is associated with hydropic abortion including various trisomies.

partial hydatidiform mole.* In these cases the placental tissue is usually of normla volume, but occasional vesicles up to 2 cms. in size can be recognised grossly or with the aid of a hand lens. Microscopically there is also hydropic change, often with central cisternal formation and focal trophoblastic hyperplasia around the circumference of the whole villus. Most of these cases are now known to be of triploid chromosomal constitution, XXY or XXX.

classical hydatidiform mole.* This is characterised by obvious gross vesicular transformation of the placental tissue, and microscopically there is severe hydropic change with variable trophoblastic proliferation. Complete moles have now been shown to have a 46 XX chromosome constitution of androgenetic origin.

* C.W. Elston, 1982 (personal comminication)

APPENDIX 5: PROTOCOL FOR THE REQUEST OF A DEVELOPMENTAL POSTMORTEM.

OBSTETRICAL HISTORY

Previous pregnancies:	sex	gestation	birth weight	eventual pathology
1	...	.....	.....	.....
2	...	.....	.....	.....
3	...	.....	.....	.....

more than 3 .....

Present pregnancy:

gestational age: .. weeks (certain/uncertain)
complications (haemorrhage-hypertension etc.):

supplementary examinations (AFP-HPL-Ultrasound-Amniocentesis etc.):

Maternal diseases (incl. smoking, alcohol, drugs):

Family history (genetic disease, malformations etc.):

DELIVERY

I.U.D.: time death .... weeks gestation
time delivery .... weeks

Presentation:

Membrane rupture (spontaneous-premature): .... hours/days/weeks

Delivery (induction-operation-fetal stress etc.):

Birthweight: .... gr. Crown-heel:....cm Occipitofrontal circumf.:.....cm

Apgar score: $pH_{v.umb.}$: $pH_{art.umb.}$: BPD:

Resuscitation:

NEONATAL HISTORY

Hypo-hyperthermia :

Assisted ventilation:

Catheter/drainage :

Other examination/ treatments :

Parenteral feeding :

Surgery:

Supplementary tests already requested :

Clinical diagnosis :

Special requests :

APPENDIX 6: GROSS EXAMINATION-ANTHROPOMETRY

P.M. NR.:

NAME:

D.O.B.:

POSTNATAL AGE: YEARS
MONTHS
WEEKS
DAYS
HOURS

GESTATIONAL AGE (WEEKS)

	OBSERVED	PERCENTILE FOR GEST. AGE	PERCENTILE FOR BIRTH WEIGHT	CONCLUSION	COMMENTS
BIRTH WEIGHT					
CROWN-HEEL					
CROWN-RUMP					
O.F.C.					
CHEST CIRCUMF.					
ABDOMINAL CIRC.					
FOOTHLENGTH					
FEMURLENGTH(X-RAY)					
THYMUS					
HEART					
LEFT LUNG					
RIGHT LUNG					
LIVER					
SPLEEN					
LEFT ADRENAL					
RIGHT ADRENAL					
LEFT KIDNEY					
RIGHT KIDNEY					
BRAIN					
OTHER					

PLACENTA: WEIGHT: MAX.DIAMETER: MAX.THICKNESS:

UMBIL.CORD: LENGTH: VESSELS: DIAMETER: INSERTION: CENTRAL MARGINAL VELAMENTOUS

	COMMENTS
UMBILICAL CORD	
MEMBRANES	
FOETAL SIDE	
MATERNAL SIDE	

SUPPLEMENTARY STUDIES	COMMENTS
X-RAY	
URINE	
BLOOD	
CSF	
CHROMOSOMES	
OTHER	

APPENDIX 7: Protocol for the postmortem examination of congenital heart disease

A complete postmortem must be performed in all cases. In case of referal send heart-lung-liver bloc in toto, including infrahepatic inferior vena cava, summary of clinical/postmortem findings and cardiac-surgery report in operated cases.

I. General gross examination

Assessment abdominal viscera situs:

a) position and/or number of:
liver
gallbladder
duodenum
pancreas
spleen: - splenunculi (spleen + accesory spleens): 10/15% neonatal pm_s
- polysplenia (multiple splenunculi on both side of dorsal mesogastrium)

b) intestines: malrotation?

Abnormal heterotaxia frequently combined to abnormal thoracic situs and C.H.D.

Visceral symmetry syndromes: abdominal visceral heterotaxia + dextroisomerism + asplenia / levoisomerism + polysplenia

II. Gross examination heart-lung-liver bloc

Position cardiac apex: dextrocardia / mesocardia / levocardia

Venous systems: a) systemic i.e.: 1) drainage persistent left superior vena cava into:
a) coronary sinus
b) left atrium: search for complex CHD often associated with the visceral asymmetry syndroms
2) drainage infrahepatic i.v.c. via azygos system into s.v.c.

b) pulmonary i.e.: increased heart mobility in cases with abnormal pulmonary venous connections

Juxtaposition atrial appendages - both appendages on same side arterial pedicle

Coronary arteries: indicate position of septum therefore determining position/size of ventricles

Relationship between pulmonary and aorta

Ductus arteriosus: Inspect position and size. At a later stage with dissection look at aortic and pulmonic sites but do not probe!

Further assessment (including lumen) must be carried out in the block taken for histology.

III. Dissection heart-lung-liver bloc

It is advisable to perform this dissection after fixation.

a) Assessment of thoracic situs

situs: solitus / isomeric / inversus

- lungs lobulation
- bronchial structure ►
  - short towards horizontal ► morph right
  - long towards vertical ► morph left
- bronchoarterial relationship ►
  - eparterial ► morph. right
  - hyparterial ► morph left
- morphology atrial appendages ►
  - broad pedicle blunt ► morph. right
  - narrow pedicle hooked ► morph. left

Chambers are identified by their morphology (morphologically right or morphologically left) regardless their position.

b) Assessment atrioventricular connection

1) Univentricular av connection
2) Biventricular av connection
   - concordant i.e.: right atrium right ventricle
   - discordant i.e.: right atrium left ventricle
   - isomeric i.e.: isomeric atria ventricle

Double inlet av connection both atria to same ventricle

Absence av connection a) imperforate valve not considered as absence of av connection

b) absence not to be considered as common av valve

c) Assessment av valves morphology/position

Imperforate av valve

Common av valve - one av valve for 2 atria communicating with ventricle(s) in the form of a) univentricular b) biventricular av connection

Straddling av valve - tension apparatus attached both sides ventricular septum

Overriding av valve - ostium overrides an underlying ventricular septum

- 50% rule - av valve belongs to ventricle receiving 50% or more of the overriding/ straddling av valve.
This may change the type of connection from biventricular to univentricular and viceversa

d) Assessment of ventricles

Ventricle: each and every chamber between the atria and the great arteries

Dominant ventricle: Ventricular mass receiving both av inlets, including the major part (> 50%) of an overriding ostium or a straddler.

Rudimentary ventricle: Ventricular mass not receiving an inlet (av valve) or less than 50% of a straddling/overriding valve

e) Assessment of great arteries connections

Concordant -	R.V./P.A.	congenitally corrected	with	disconcordant av connection
Discordant -	R.V./aorta - transposition	complete	with	concordant av connection

Double outlet - both arteries from same ventricle

Single outlet - a) truncus arteriosus
b) pulmonary atresia
c) aortic atresia

Overriding arterial valves also follow 50% rule

IV.Histology

Blocs from:

all lung lobes

all heart chambers (including ductus arteriosus)

right/left liver lobes

kidneys

brain (cortex/basal ganglia-ventricle/hippocampus/mesencephalon/pons medulla oblongata/cerebellum).

conduction system (when indicated) Med. Lab. Sci. 34: 223: 1977

lungs evaluation of

- plexogenic pulmonary arteriopathy
  - I medical hypertrophy
  - II intimal proliferation
  - III intimal fibrosis
  - IV plexiform lesions
  - V dilatation lesions
  - VI necrotizing arteriitis
- pulmonary venous hypertension – "arterialization" of pulmonary veins

APPENDIX 8: HISTOLOGY FORM P.M. NR.:

TISSUE	BLOCK NR.	SPECIAL METHODS-COMMENTS
THYROID-OESOPHAGUS TRACHEA		
LARYNX		
THYMUS		
LUNGS		
PERICARDIUM		
HEART		
DIAPHRAGM		
LIVER		
HILUS		
GALLBLADDER		
PANCREAS		
SPLEEN		
STOMACH		
SMALL BOWEL		
LARGE BOWEL		
MESENTERIUM		
ADRENALS		
KIDNEYS		
URETER-AORTA-VENA CAVA		
"CLOACA"		
UTERUS-CERVIX-VAGINA		
OVARIES-TUBES		
TESTES-EPIDIDYMIS		
LYMPH NODES		
SKIN		
MUSCLE		
BONE MARROW		
URACHUS-ARTERIES		
UMBILICAL VEIN		
OUCTUS ARTERIOSUS		
OTHER		

TISSUE	BLOCK NR.	SPECIAL METHODS-COMMENTS
SKELETON:		
RIBS		
"MIDDLE EAR"		
OTHER		
CNS:		
CORTEX		
VENTRICLES-BASAL GANGLIA		
HIPPOCAMPUS		
CHOROID PLEXUS		
HYPOPHYSIS		
PINEAL GLAND		
MESENCEPHALON		
PONS		
CEREBELLUM		
MEDULLA OBLONGATA		
SPINAL CORD		
MENINGES		
PERIPHERAL NERVES		
OTHER		
PLACENTA:		
UMBILICAL CORD: PROXIMAL DISTAL		
MEMBRANES "ROLL"		
INSERTION		
PLACENTA CENTRAL (NORMAL) PATHOLOGY		
PLASTIC		
EM		
IMMUNOPEROXIDASE		
MORPHOMETRY		
OTHER		

APPENDIX 9: Summary of the guidelines for performing a developmental postmortem including final report.

Fetus/stillbirth/livebirth

1. X-ray: assessment of 1) skeletal maturation (femur length)
   2) pathology - skeletal
   soft tissues

2. Photograph: anterior
   posterior
   abnormalities

3. Gross examination with anthropometric assessment:

   performed in 2 steps:

   1) fresh: organs taken out
   in blocks
   specimens for
   supplementary studies

   2) fixed: further dissection
   of organs after
   fixation
   organ weights
   standard blocks

4. Placenta:

   Gross examination after fixation
   Measurements (weight without cord/membranes)
   Standard blocks

5. Histology:

   Standard blocks Specialized techniques

6. Report:

   Summary of findings together with comments on:

   1) assessment of growth/maturation
   anthropometric assessment
   maturation assessment: X-ray (femur length -ossification centres)
   Gross examination brain
   histology: kidney

   2) assessment of pathology

   3) assessment of intensive care related changes

   4) assessment of risk factors for future pregnancies, and/or relatives

APPENDIX 10: Protocol for examing a perinatal brain.

Removal

Select technique according to available clinical data and eventual postmortem X-ray findings.

Fixation

1. Fix in 10% formaldehyde (saturated solution with NaCl) for 3 weeks
2. Weigh the brain
3. Fix in 70% alcohol for 1 week
4. Weigh the brain

Histology

1. Tissue samples are left overnight in 10% formaldehyde
2. Postfix with saturated aqueous picric acid 1:1 with 95% alcohol for 8 hours
3. Dehydrate overnight in 70% alcohol
   2 hours in 95% alcohol
   2 hours in 95% alcohol
   2 hours in 100% alcohol
   2 hours in 100% alcohol
4. Clear in xylol 1 overnight
   xylol 2 1 day
5. Mount in paraffin 1 overnight
   paraffin 2 1 day

APPENDIX II: Samples for histological examination of the perinatal central nervous system.

1. frontal cortex-white matter
2. lateral ventricle-subependymal germinal matrix-basal ganglia and thalamus area
3. hippocampus
4. midbrain
5. pons
6. medulla at level of olives
7. cerebellar hemisphere with dentate nucleous
8. cervical spinal cord
9. thoracic spinal cord
10. lumbar spinal cord
11. choroid plexus
12. meninges
13. muscle (enzyme histochemistry ► on selective basis
14. eye

APPENDIX 12: Morphological classification of brain lesions with reference to brain imaging grades.

Grade 0 (no haemorrhage)

Grade 1 (only GMH[1])

Grade 2 (GMH or CPH[2] with IVH[3], without ventricular dilatation)

Grade 3 (GMH or CPH with IVH and ventricular dilatation)

Grade 4 (GMH or CPH with IVH and parenchymal haemorrhage)

Periventricular leucomalacia (PVL)

PVL + haemorrhage

Cerebral edema

Cyst

Subarachnoidal haemorrhage

Subdural haemorrhage

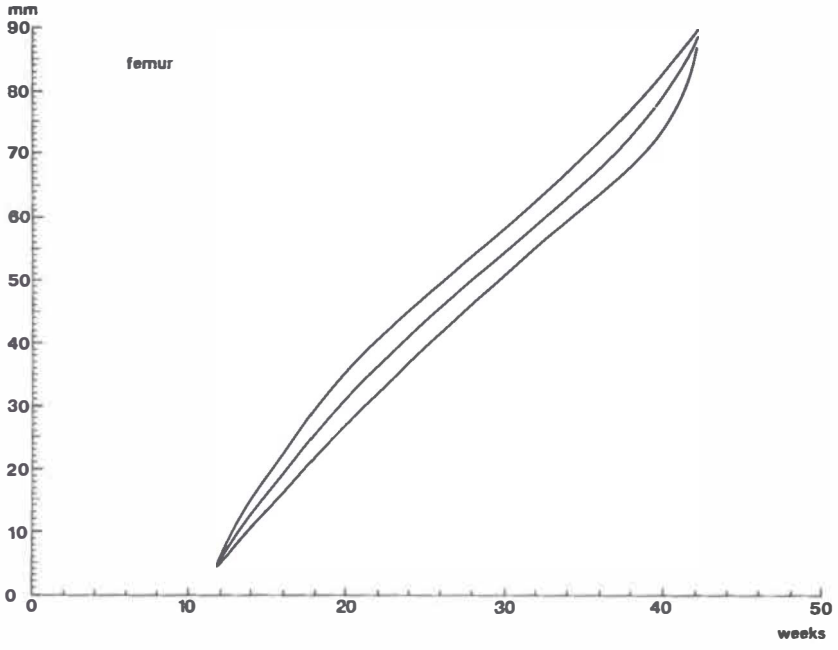
Hydrocephalus

1 germinal matrix haemorrhage

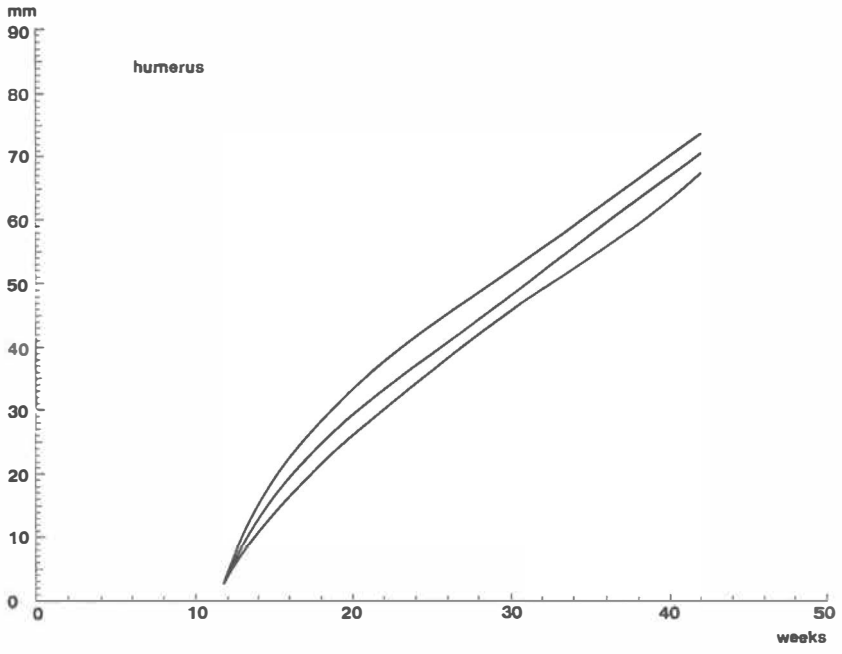
2 choroid plexus haemorrhage

3 intraventricular haemorrhage

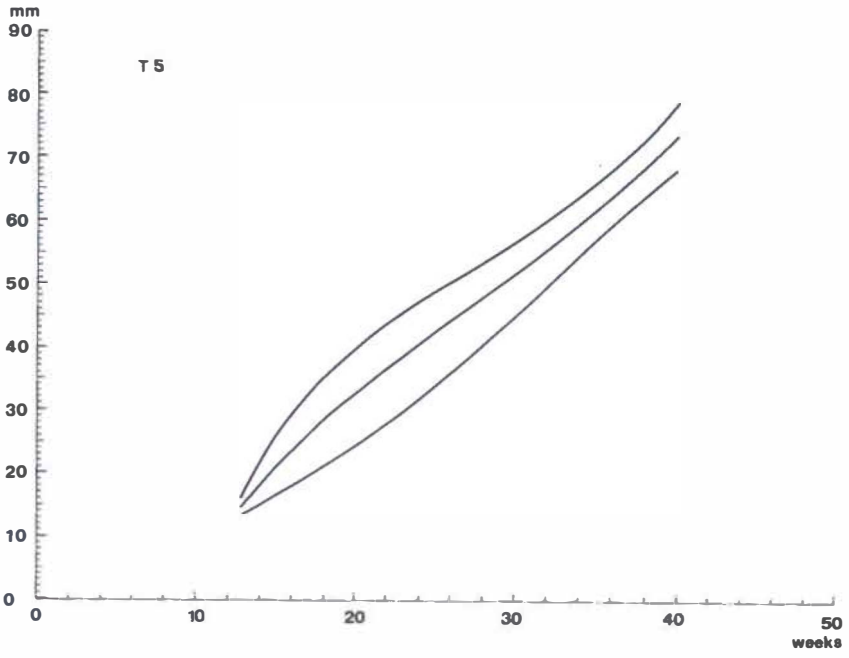
APPENDIX 13: Smoothed curve from the radiographic assessment of femur length (mean ± 2 SD) in relation to gestational age.



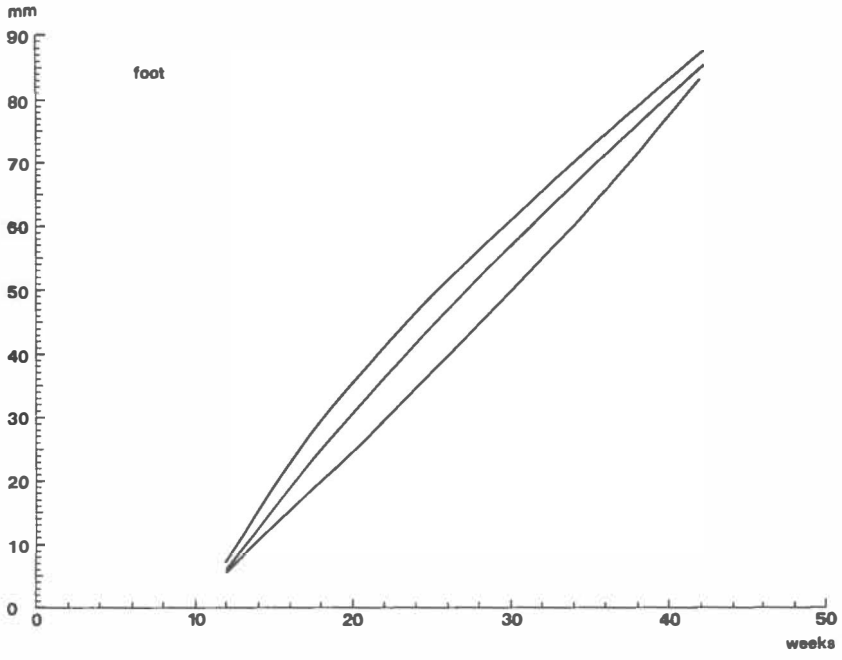
APPENDIX 14: Smoothed curve from the radiographic assessment of humerus length (mean ± 2 SD) in relation to gestational age.



APPENDIX 15: Smoothed curve from the radiographic measurement of thorax diameter at the level of T_5 (mean $\pm$ 2 SD) in relation to gestational age.



APPENDIX 16: Smoothed curve from measurement of foot length (mean ± 2 SD) in relation to gestational age.



APPENDIX 17: Guidelines for the assessment of maturity from 12 to 42 weeks of gestation

Gestational Age			
12 wks			
	X-ray (femur)		
23 wks ►	(3 layers glomeruli present)		
24			
25			
26			
27			
28	1 layer = 1 week	nephrogenic zone	
29			
30			
31			
32			
33			
34 wks ►	focal remanents of nephrogenic zone		
36 ►	no nephrogenic (approx.12 layers glomeruli)	36 pinhead femoral centre 37 femoral centre 38 tibial centre	X-ray-ossification centres
Term		40 wks ► femoral centre > tibial centre	
		42 wks ► femoral centre = tibial centre	
42 wks			

APPENDIX 18: Assessment of brain maturation on gross examination.

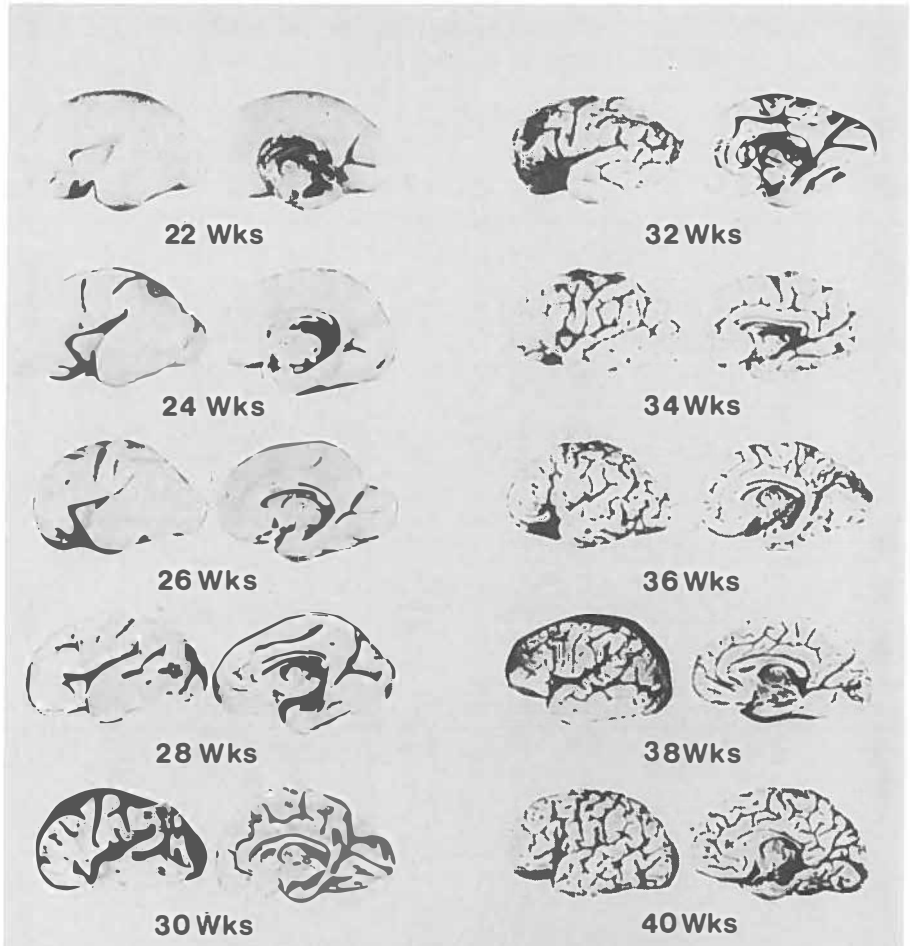


Fig 1.—Characteristic configuration of fetal brains from 22 to 40 weeks' gestation at two week intervals. All brains have been brought to same size. Explanation of markings in text

Arch. Pathol. Lab. Med. vol 101, April 1977
Gestational Development - Dorovini-Zis & Dolman.

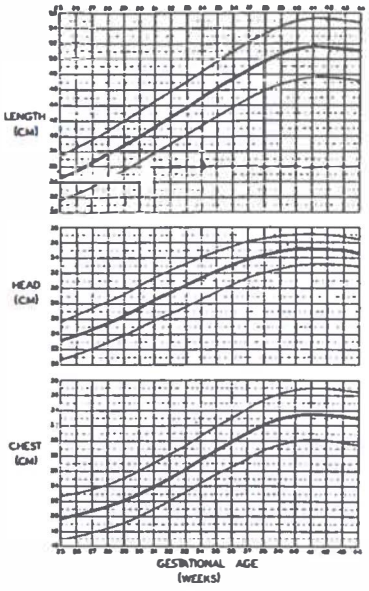
APPENDIX 19

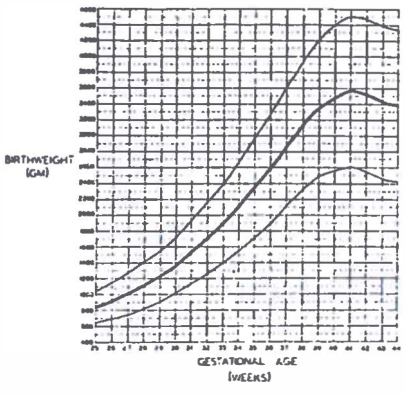
ANTHROPOMETRY

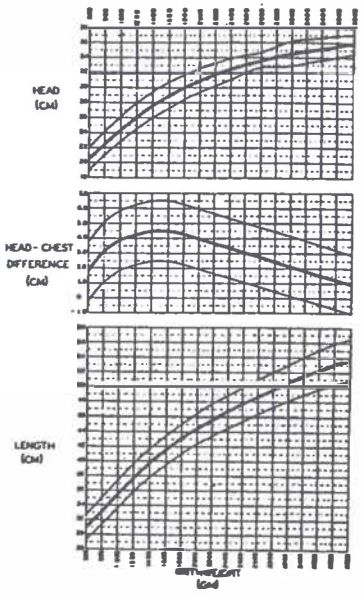
P.M. NR.:

NAME:

D.O.B.:







J. Pediatrics 74:901:1969

SUMMARY

In this thesis guidelines are established for a service in developmental pathology that can further enhance the different aspects of developmental medicine.

Such a service is primarily dependent on the routine use of a comprehensive developmental postmortem protocol for the examination of embryonic and perinatal deaths. Therefore the most important parts of the developmental postmortem, including routine postmortem radiography, assessment of maturation and growth as well as developmental neuropathology, are discussed with an emphasis on the methodology. The more relevant information for the performance of a developmental assessment of embryonic and perinatal cases is provided in the Appendix. Special stress is laid upon the evaluation of maturation and growth since many forms of fetal and neonatal pathology are closely related to the developmental stage of the individual case.

A methodological approach as proposed here represents the basis for an analysis of the causes of perinatal morbidity and mortality. This is necessary to identify those factors that might be influenced by changes in clinical management in order to achieve an improvement in perinatal care. In this context the causes of perinatal deaths in a Dutch series were surveyed by means of the outlined developmental postmortem protocol. Furthermore a model for a perinatal audit is presented together with the results obtained from its application to the study of two Scandinavian populations. The results of the Norwegian perinatal audit indicate that nearly one-third of the perinatal deaths might be avoided without any great injection of new resources in terms of personnel and equipment. It is therefore suggested that regional perinatal audits are established in order to inquire into possible avoidable factors that will help to further reduce both perinatal morbidity and mortality.

Recent developments around antenatal diagnosis and surveillance as well as emerging therapeutical possibilities in utero have resulted in an increasing need for a detailed morphological examination of embryonic and fetal deaths. The postmortem findings reported in this thesis further underline the importance of a routine examination of the different types of early pregnancy loss. Moreover it demonstrates that hypoxic-ischaemia must be considered as an equally important factor

together with anomalies, chromosomal abnormalities and genetic disease in the evaluation of the pathology of early pregnancy.
These results indicate that an optimal antenatal diagnosis and surveillance depends on a close collaboration between clinicians and pathologists.

The introduction of modern ultrasound allows for the assessment of both fetal/neonatal anatomy as well as function (e.g. fetal movements, fetal-placental haemodynamics). Therefore the interpretation of the morphological findings at postmortem in relationship with the functional status during life is what one has here defined as functional morphology. This is well illustrated by the comparison between fetal movement patterns and morphological findings in the study of abnormal motor behaviour in anencephalic. Our results obtained from correlating postmortem anatomical and sonographic findings in brains of newborns with haemorrhagic and/or ischaemic pathology further underline the advantages of such an approach.

Special attention is given to the placenta because the morphological examination of this fetal organ is very much part of a developmental assessment. The placenta pathology in insulin-dependent diabetic patients treated with continuous subcutaneous insulin infusion (CSII) emphasizes the role of the placenta in the understanding of pregnancy associated pathology. In addition it also demonstrates that deviations in the developmental stage of the placenta can represent an important source of pathology as is the case for a number of fetal and neonatal conditions.
Furthermore this study demonstrates that a tight glycaemic control achieved with CSII does not affect the morphological expression of diabetes in pregnancy.

Our results with the use of the pregnant Wistar rat indicate that this is a useful experimental animal model for the study of the pathology of uteroplacental circulation in haemochorial placentation. Therefore this model can be used to evaluate fetal growth retardation. For this purpose the radiographic assessment of fetal growth as presented here represents a simple and reliable method that also allows for the detection of eventual skeletal anomalies.
On the basis of our findings with this animal model we propose that early growth delay seen in diabetes is the result of an abnormal

uteroplacental circulation.

The absence of malformations among the treated diabetic rats (streptozotocin insulin pump) in our experimental model suggests that the teratogenic effect of diabetes is not only dependent on a time in gestation but also on a prolonged and substantial disturbance of the glucose blood level.

SAMENVATTING

Voor een goede prenatale en perinatale zorg is ontwikkelingspathologie onontbeerlijk. Om een goede service te kunnen verlenen maakt de ontwikkelingspatholoog gebruik van een gedetailleerd protocol voor embryonale, foetale en perinatale obducties. De belangrijkste onderdelen van een dergelijk obductieprotocol worden in dit proefschrift beschreven. Gezien het belang van routine postmortaal röntgenonderzoek, de bepaling van maturatie en groei alsmede ontwikkelingsneuropathologie worden deze onderdelen van het obductieprotocol afzonderlijk behandeld. Nadere informatie hieromtrent wordt in de appendix beschreven.

Met nadruk wordt gesteld dat een goede evaluatie van maturatie en groei belangrijk is, omdat vele afwijkingen in de foetale en neonatale pathologie nauw samenhangen met het ontwikkelingsstadium van foetus of kind.

De in dit proefschrift beschreven methoden vormen de basis voor een goede analyse van de oorzaken van perinatale morbiditeit en mortaliteit. Hierdoor kunnen factoren worden herkend welke van belang kunnen zijn voor de noodzakelijke planning van de perinatale zorg, zodat de overlevingskansen en de morbiditeit kunnen worden verbeterd.

Met dit in gedachten werden de oorzaken van perinatale sterfte in een Nederlandse serie onderzocht, waarbij het beschreven obductieprotocol werd gebruikt. Voorts is een model voor "perinatale audit" ontworpen. Tevens worden de resultaten beschreven welke werden verkregen nadat dit model werd gebruikt in een onderzoek van twee Scandinavische series. De conclusie van het Noorse perinatale audit is dat bijna eenderde deel van de gevallen van perinatale sterfte kan worden voorkomen zonder dat uitbreiding nodig is van personeel of apparatuur. Voor een kritische beoordeling van perinatale morbiditeit en mortaliteit is het instituut van de "perinatale audit" derhalve onmisbaar.

Door de recente ontwikkelingen, zoals antenatale diagnostiek en bewaking alsmede de nieuwe nog niet volledig ontwikkelde therapeutische mogelijkheden in utero, is een toegenomen behoefte aan gedetailleerd morfologisch onderzoek van embryo en foetus ontstaan. De postmortale bevindingen welke in dit proefschrift worden beschreven, onderstrepen nogmaals het belang van een routinematig onderzoek van vroege, spontane en therapeutische, zwangerschapsonderbreking. Hypoxische-ischaemische

veranderingen zijn een belangrijk onderdeel van de pathologie van de vroege zwangerschap, evenzeer als congenitale afwijkingen, chromosomale afwijkingen en erfelijke ziekten.

Deze resultaten tonen dat een optimale antenatale diagnostiek en bewaking afhankelijk is van een nauwe samenwerking tussen klinici en pathologen.

Functionele morfologie bestudeert het verband tussen de morfologische bevindingen bij de obductie en de functionele status tijdens het leven. Met behulp van het huidig echografisch onderzoek kan zowel de foetale-neonatale anatomie als de foetale-neonatale functie (b.v. bewegingspatronen en foetale-placentaire haemodynamica) worden beoordeeld. Een goed voorbeeld is de relatie van foetale bewegingspatronen met morfologische bevindingen bij anencephalie, waarbij men abnormale bewegingspatronen aan kan treffen. Een ander voorbeeld is de relatie tussen postmortale afwijkingen met echografische bevindingen bij ischaemische haemorrhagische hersenlaesies bij pasgeborenen.

Veel aandacht wordt besteed aan de placenta, omdat het morfologisch onderzoek van dit foetaal orgaan belangrijk is voor de ontwikkelingspathologie. De afwijkingen in de placenta bij insuline-afhankelijke diabetische patienten, welke worden behandeld met continue subcutane insuline infusie (CSII) geven nogmaals aan dat het onderzoek van de placenta van belang is voor het kunnen begrijpen van de pathologie tijdens de zwangerschap. Tevens wordt aangetoond dat dysmaturiteit van de placenta een belangrijke bevinding kan zijn bij diverse foetale en neonatale afwijkingen.

Aangetoond is dat controle van de bloedsuikerspiegels met behulp van CSII niet van invloed is op de morfologische afwijkingen bij zwangerschapsdiabetes.

De zwangere Wistar rat blijkt in onze studie een goed proefdiermodel te zijn voor het bestuderen van veranderingen in de uteroplacentaire circulatie bij haemochoriale placentatie. Derhalve kan dit model worden gebruikt voor de evaluatie van foetale groeivertraging. Voor dit doel is radiologisch onderzoek van foetale groei, zoals in dit proefschrift is beschreven, een eenvoudige en betrouwbare methode, waarmee tevens eventuele skeletafwijkingen kunnen worden aangetoond. Op grond van onze resultaten met behulp van dit proefdiermodel is onze hypothese dat vroege groei-achterstand bij diabetes het gevolg is van een abnormale

utero-placentaire circulatie.

De afwezigheid van congenitale afwijkingen bij behandelde diabetische ratten (streptozotocine en insulinepomp) in ons proefdiermodel suggereert dat het teratogene effect van diabetes niet uitsluitend afhankelijk is van een bepaalde periode tijdens de zwangerschap maar tevens van langdurige verstoring van de bloedsuikerspiegel.